Addiction Biology

INVITED REVIEW

doi:10.1111/adb.12204

Common single nucleotide variants underlying drug addiction: more than a decade of research

Kora-Mareen Bühler[1], Elena Giné[2], Victor Echeverry-Alzate[1], Javier Calleja-Conde[1], Fernando Rodriguez de Fonseca[3] & Jose Antonio López-Moreno[1]

Department of Psychobiology, School of Psychology[1] and Department of Cellular Biology, School of Medicine,[2] Complutense University of Madrid, Fundación IMABIS,[3] Laboratorio de medicina Regenerativa, Hospital Regional Universitario Carlos Haya, Málaga, Spain

ABSTRACT

Drug-related phenotypes are common complex and highly heritable traits. In the last few years, candidate gene (CGAS) and genome-wide association studies (GWAS) have identified a huge number of single nucleotide polymorphisms (SNPs) associated with drug use, abuse or dependence, mainly related to alcohol or nicotine. Nevertheless, few of these associations have been replicated in independent studies. The aim of this study was to provide a review of the SNPs that have been most significantly associated with alcohol-, nicotine-, cannabis- and cocaine-related phenotypes in humans between the years of 2000 and 2012. To this end, we selected CGAS, GWAS, family-based association and case-only studies published in peer-reviewed international scientific journals (using the PubMed/MEDLINE and Addiction GWAS Resource databases) in which a significant association was reported. A total of 371 studies fit the search criteria. We then filtered SNPs with at least one replication study and performed meta-analysis of the significance of the associations. SNPs in the alcohol metabolizing genes, in the cholinergic gene cluster *CHRNA5-CHRNA3-CHRNB4*, and in the *DRD2* and *ANNK1* genes, are, to date, the most replicated and significant gene variants associated with alcohol- and nicotine-related phenotypes. In the case of cannabis and cocaine, a far fewer number of studies and replications have been reported, indicating either a need for further investigation or that the genetics of cannabis/cocaine addiction are more elusive. This review brings a global state-of-the-art vision of the behavioral genetics of addiction and collaborates on formulation of new hypothesis to guide future work.

Keywords Candidate gene association studies, drug addiction, GWAS, single nucleotide polymorphisms.

Correspondence to: Jose Antonio López-Moreno, Department of Psychobiology, School of Psychology, Complutense University of Madrid, Campus de Somosaguas, Madrid 28223, Spain. E-mail: jalopezm@psi.ucm.es

INTRODUCTION

Like most psychiatric disorders, drug addiction is a common complex disease determined by multiple factors, including genetics. Family, adoption and twin studies have estimated that genetic vulnerability (i.e. the susceptibility to develop a disease because of inherited-genetic components) to drug addiction may be as high as 50 percent (Duaux *et al.* 2000; Ball 2008). Nevertheless, few addiction-risk or protective genes have been validated, and the genetic architecture of drug addiction remains elusive.

Inheritance of a common complex disease is not attributable to a mutation in one single gene (as in a classical Mendelian disorder) but to a combination of effects of several different gene variants (Lander & Schork 1994). A major part of these gene variants are common, with each having only a small individual effect on disease risk. These common low-effect variants are also called polymorphisms. Polymorphisms are defined as DNA sequence variations that produce more than one alternate form of a particular gene (i.e. alleles) within the population, with the least common allele having a frequency (minor allele frequency—MAF) of a minimum of 1 percent in the population (Twyman & Primrose 2003). The most frequent types of polymorphism are single nucleotide polymorphisms (SNPs), i.e. variants of DNA sequences that differ in one single nucleotide base. Human SNPs are nearly always bi-allelic (although, in principle, an SNP could also be tri- or tetra-allelic), with one allele possessing the ancestral or more common nucleotide, and a second allele in which this ancestral nucleotide is substituted by another. The consequences of such a nucleotide substitution on a gene depend on its

location in the DNA sequence (i.e. within or outside a protein coding region) and if the substitution affects or not the encoded amino acid. Therefore, SNPs within a coding region may be divided into two main categories: synonymous or silent SNPs, in which the variant allele encodes for the same amino acid and does not affect the protein configuration, and non-synonymous SNPs, in which the variant allele encodes for a different amino acid and affects the protein configuration. Some SNPs affect gene regulation, gene expression or messenger RNA (mRNA) product and occasionally protein function. In these cases, SNPs are referred to as 'functional SNPs' (Mottagui-Tabar *et al.* 2005; Albert 2011). Nevertheless, most SNPs are neutral and have no effect on phenotype (Ramensky, Bork & Sunyaev 2002; Johnson 2009). SNPs are highly abundant in the human genome with over 15 million having been identified (Altshuler *et al.* 2010). Many of them, mainly the non-synonymous SNPs, are believed to constitute the genetic background of phenotypic variation among individuals (Ramensky *et al.* 2002), including vulnerability to diseases such as drug addiction.

An important property of SNPs is that they can also be grouped by linkage disequilibrium (LD). LD refers to the fact that SNPs that are physically close in the chromosome will be inherited together, and their occurrence will be correlated with each other (Syvänen 2001; Frazer *et al.* 2009). LD is especially useful because it allows for the division of the genome into groups of correlated SNPs. By genotyping just one SNP of each group ('tag'-SNP), information regarding over 80 percent of all SNPs at a MAF of higher than 5 percent can be gained (Frazer *et al.* 2009). As described below, genome-wide association studies (GWAS) rely on this linkage pattern between SNPs.

The two main approaches in genetic association studies used to identify disease-risk gene variants are candidate gene association studies (CGAS) and GWAS. CGAS focus on the selection of genes that were previously linked to the disease and are therefore 'candidate-genes' for participating in the biological mechanism underlying the disease. SNPs with a functional consequence on the gene, for instance, affecting its regulation or protein product, are selected and assessed for association with the disease. Frequently, this process follows a case–control design, although family-based designs may also be used (Hirschhorn *et al.* 2002; Patnala, Clements & Batra 2013). On the contrary, GWAS directly assesses hundreds of thousands of tag-SNPs throughout the genome to detect differences in SNP allele frequencies between a series of disease-affected cases and unaffected controls. In comparison to CGAS, GWAS are hypothesis-free, meaning that no specific candidate gene is being tested. As a result, most genes identified through GWAS have not been previously related to the disease under investigation (Frazer *et al.* 2009).

Addiction genetics research has extensively used CGAS and GWAS approaches to identify and test potential risk or protective gene variants. A large number of CGAS and several GWAS studies, mostly covering alcohol and nicotine addiction, have been published in recent years, and first GWAS of cannabis and cocaine addiction are now available (Agrawal *et al.* 2011; Gelernter *et al.* 2014a; for reviews on association studies on substance dependence, see Li & Burmeister 2009 and Treutlein & Rietschel 2011). These studies have provided many SNP-addiction phenotype associations, although with an important lack of validation or replication (the issue of replication failure will be addressed further in the Discussion section).

The purpose of the present paper is to provide a broad meta-analysis and review of the SNPs most significantly associated with alcohol-, nicotine-, cannabis- and cocaine-related phenotypes, in family-based, case-only or case–control candidates from CGAS and GWAS published between the years of 2000 and 2012. We first filtered and described the SNPs associated with each drug, performed meta-analysis on replicated SNPs, selected the most significant SNPs and finally performed a literature review of the biological implication of these SNPs. This paper aims to synthesize the results of the whole typology of association studies regarding drug dependencies to provide a comprehensive overview of the implication of SNPs in alcohol, nicotine, cannabis and cocaine dependence.

MATERIALS AND METHODS

Literature search and inclusion criteria

To identify significant published associations between alcohol-, nicotine-, cannabis- and cocaine-related phenotypes and SNPs, we performed a review of the literature from 2000 through 2012 using the PubMed/MEDLINE database (National Center for Biotechnology Information, NCBI) and the Addiction GWAS Resource (AGR, http://addictiongwas.com). For the PubMed/MEDLINE search strategy, keywords were set as follows: alcoholAND('dependence'OR'addiction')AND('polymorphism'OR'snp'); alcoholismAND('polymorphism'OR'snp'); nicotineAND('dependence'OR'addiction')AND('polymorphism'OR'snp'); cannabisAND('dependence'OR'addiction')AND('polymorphism'OR'snp'); cocaine AND('dependence'OR'addiction')AND('polymorphism'OR'snp'); publications were limited to the human species and data range was set from January 1, 2000 to December 31, 2012.

Studies were considered eligible if they met the following criteria: (1) the study was an original research article

published in a peer-reviewed English language international scientific journal; (2) the study was a family-based, case-only or case–control CGAS or GWAS; (3) the study revealed an association between an SNP and an alcohol-, nicotine-, cannabis- or cocaine-related phenotype (see Table 1 for description of the specific phenotypes); (4) the SNP was statistically significantly associated with the phenotype (nominal *P*-value was < 0.05); and (5) the study participants had no other co-morbid disease and were not polydrug abuser (except for alcohol and nicotine co-abuse), or this condition was at least controlled in the association analysis.

Table 1 Addiction phenotypes included in this review.

Addiction phenotypes	
Alcohol	Alcohol addiction and/or dependence and abuse (as well as related measures), alcohol withdrawal symptoms and craving, age of onset of alcohol dependence, heavy alcohol consumption, alcohol consumption and use
Nicotine	Nicotine addiction and/or dependence and abuse (as well as related measures), nicotine withdrawal symptoms and craving, age of onset of regular smoking, smoking behavior, cigarette consumption and use
Cannabis	Cannabis addiction and/or dependence and abuse (as well as related measures), cannabis withdrawal symptoms and craving, age of onset, cannabis consumption and use
Cocaine	Cocaine addiction and/or dependence and abuse (as well as related measures), cocaine withdrawal symptoms and craving, age of onset, cocaine consumption and use

Data extraction and study selection

The following data were extracted from each eligible study: (1) gene; (2) SNP rs number or nucleotide variation; (3) authors; (4) year of publication; (5) type of study; (6) sample size; (7) study population ethnicity or nationality; (8) explored phenotype and diagnostic or assessment tool; (9) main finding; and (10) *P*-value and effect size (if available) of the association.

Study identification and selection

The flow of study identification and selection is shown in Fig. 1. The PubMed Database and AGR search strategy retrieved 2102 potentially relevant studies. After title and abstract revision, 1690 papers were excluded because they did not meet one or more of the inclusion criteria, leaving 411 papers for full-text article assessment. Of these 411 papers, 17 were excluded because the full texts were not accessible and abstracts did not provide enough information to extract the required data (for reference, see Supporting Information Table S1). Furthermore, 15 articles were excluded because a significant association was only found between an SNP haplotype and a phenotype without an SNP association (for reference, see Supporting Information Table S1). The study of Rommelspacher *et al.* (2001) on alcohol dependence as well as the study of Luo *et al.* (2003) on cocaine dependence were excluded because a full-text review of these articles showed that association with the phenotype of interest only trended toward significance (Rommelspacher *et al.* 2001), or the association was only significant with a combination of other abused drugs (alcohol and opioids; Luo *et al.* 2003). Four other articles

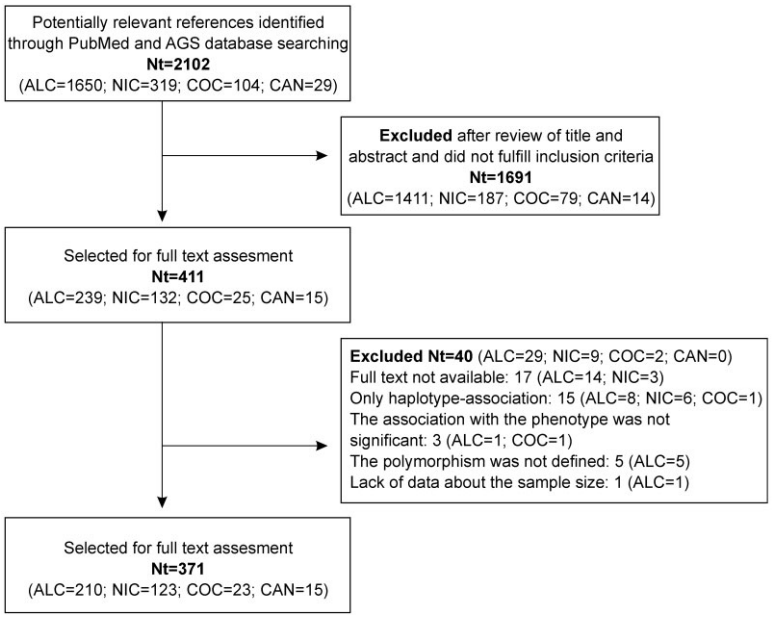

Figure 1 Flowchart of the selection of alcohol-, nicotine-, cocaine- and cannabis-related studies. AGS = Addiction GWAS Resource; ALC = alcohol papers; CAN = cannabis papers; COC = cocaine papers; NIC = nicotine papers; Nt = total number of research papers

were excluded from the review because the polymorphism explored in these studies were not defined as an SNP (Loh *et al.* 2000; Montano Loza *et al.* 2006) or no rs number from dbSNP or nucleotide variation was provided (Park *et al.* 2006; Wei *et al.* 2012). The paper of Reimers *et al.* (2012) was excluded because they studied the association between alcohol dependence and complete gene sets, as opposed to single gene or SNP associations. The paper of Edenberg *et al.* (2004) was excluded because it did not provide the specific sample size of the study population.

As a result, 371 studies were included for meta-analysis and review: 210 for alcohol-related phenotypes, 123 for nicotine-related phenotypes, 15 for cannabis-related phenotypes and 23 for cocaine-related phenotypes. Data extracted from these studies are presented in Supporting Information Table S2 for alcohol, Supporting Information Table S3 for nicotine, Supporting Information Table S4 for cannabis and Supporting Information Table S5 for cocaine.

Despite the efforts made to be complete, we are aware of the possibility that some association studies might not have been included in our review. However, we believe that this review is a suitable accurate representation of the published studies investigating the association between SNP and alcohol, nicotine, cannabis and cocaine phenotypes.

Combination of *P*-values of replicated studies and SNP selection

If an association between an SNP and an alcohol-, nicotine-, cocaine- and cannabis-related phenotype has been replicated once or more in the included studies, Fisher's method for combining *P*-values was performed (Fisher, Immer & Tedin 1932) to carry out a joint analysis of the significance of the association. SNPs were than sorted according to their combined *P*-values, and the most significant SNPs related to each drug were selected for literature review.

Linkage disequilibrium analysis

Finally, we performed pairwise LD of the selected SNPs using the web-based tool SNP Annotation and Proxy Search (SNAP) from the Broad Institute (Johnson *et al.* 2008). Please note that because this study aims to give an overview of the most significant, and also most replicated, associated SNPs, we described every SNPs independently, despite the fact that some were in high LD (because different studies treated different SNPs).

RESULTS

In the following sections, we will focus on the SNPs in our meta-analysis most significantly associated with alcohol-, nicotine-, cocaine- and cannabis-related phenotypes, examining their characteristics, functional consequences in the protein (if any) and their relation to drug addiction.

SNP and ALCOHOL-related phenotypes

From the 210 studies on alcohol-related phenotypes, we retrieved significant associations from 772 SNPs tagging 301 different genes, in addition to 138 SNPs located in non-genic regions. Table 2 shows the 20 most significant SNPs associated with alcohol-related phenotypes. Of these SNPs, the most replicated belong to alcohol-metabolizing enzymes and components of different neurotransmitter systems. In this section, these SNPs will be examined in detail.

Variants in genes encoding for alcohol-metabolizing enzymes

The two most significant SNPs, which are also the subject of the most replication studies, belong to the genes encoding alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes. These enzymes compose the main pathway of alcohol metabolism, converting alcohol into acetaldehyde and acetaldehyde into acetate.

Seven genes encode the different ADH enzymes, and three genes encode the ALDH enzymes. The seven *ADH* genes are localized on chromosome 4 in a segment of approximately 370 kb (Han *et al.* 2007). While the class 1 ADH enzymes (ADH1A, ADH1B and ADH1C) play a major role in oxidative alcohol metabolism in the liver, ADH4 is predominantly expressed in the epithelial tissues of the upper digestive tract (esophagus, gingival and tongue), stomach and liver (Yin *et al.* 2003; Wei *et al.* 2012) and contributes to the presence of higher levels of alcohol consumption. ADH7 has been implicated in alcohol metabolism at the level of gastroesophageal tissue before ingested alcohol reaches the liver (Hurley & Edenberg 2012). The roles of ADH5 and ADH6 are less known (for reviews of the ADH enzyme family and its involvement in alcohol metabolism, see Hurley & Edenberg 2012; Edenberg 2007).

The *ADH1B* gene (located at chromosome 4q21–q23) contains the functional-SNP rs1229984 at exon 3. This SNP encodes two different alleles at this gene: allele *ADH1B*1*, the ancestral or reference allele, which possesses the nucleotide guanine (G), and allele *ADH1B*2*, which contains the nucleotide adenine (A). In East Asians, the *ADH1B*2* allele is relatively common and up to 70–90 percent of the population carries this allele. This sentences now should read: In this context, the presence of a single *ADH1B*2* allele significantly reduces the risk for alcohol dependence in comparison to the presence of the *ADH1B*1* allele in homozygosis Goedde *et al.*

Table 2 Most significantly associated SNPs with alcohol-related phenotypes.

SNP	*Gene*	*No. of studies*[a]	*Combined P-value*	*SNP function*	*Allele change*	*Minor allele*	*mRNA position*	*AA change*	*Protein position*	*Chr.*	*Protein function*	*Effect*[b]	*LD bin*[c]
rs671	*ALDH2*	18	1.13×10^{-75}	Non-syn, Exon 12	G to A	A	1606	Glu to Lys	504	12	Enzyme	Protective	
rs1229984	*ADH1B*	27	7.05×10^{-60}	Non-syn, Exon 3	G to A	G	227	Arg to His	48	4	Enzyme	Protective	1
rs1800497	*ANKK1/DRD2*[d]	12	3.20×10^{-12}	Non-syn, Exon 8	C to T	T	2231	Glu to Lys	713	11	Enzyme/Receptor	Risk	
rs750338	*PKNOX2*	3	1.82×10^{-12}	Intron	C to T	C	NA	NA	NA	11	Transcription factor	Risk	5
rs16139	*NPY*	2	1.45×10^{-10}	Non-syn, Exon 2	A to G	G	108	Leu to Pro	7	7	Neuropeptide	Risk/Protective	
rs10893366	*PKNOX2*	2	7.20×10^{-11}	Intron	C to T	T	NA	NA	NA	11	Transcription factor	Risk	3, 4
rs10893365	*PKNOX2*	2	2.94×10^{-09}	Intron	C to T	C	NA	NA	NA	11	Transcription factor	Risk	4
rs279858	*GABRA2*	7	2.88×10^{-08}	Synon, Exon 5	A to G	G	1069	Lys to Lys	132	4	Receptor	Risk	
rs1693482	*ADH1C*	4	5.93×10^{-08}	Non-syn, Exon 6	C to T	T	1185	Arg to Gln	272	4	Enzyme	Protective	
rs698	*ADH1C*	7	2.78×10^{-07}	Non-syn, Exon 8	A to G	A	1133	Ile to Val	350	4	Enzyme	Protective	1
rs1799971	*OPRM1*	8	5.87×10^{-07}	Non-syn, Exon 1	A to G	G	252	Asn to Asp	40	6	Receptor	Risk	
rs1800759	*ADH4*	5	9.38×10^{-07}	Near Gene-5	A to C	A	NA	NA	NA	4	Enzyme	Protective	2
rs3762894	*ADH4*	4	1.94×10^{-06}	Near Gene-5	C to T	C/T[e]	NA	NA	NA	4	Enzyme	Protective	1, 5
rs701492	*GAD1*	2	1.76×10^{-05}	Intron	C to T	T	NA	NA	NA	2	Enzyme	NA	
rs1042364	*ADH4*	4	6.31×10^{-05}	Non-syn, Exon 9	A to G	A	1238	Gly to Arg	388	4	Enzyme	Protective	2, 3
rs4680	*COMT*	4	0.000156	Non-syn, Exon 4	G to A	A	452/696[f]	Val to Met	108/158[f]	22	Enzyme	Risk/Protective	
rs16944	*IL1B*	2	0.000170	near Gene-5	A to G	A	NA	NA	NA	2	Cytokine	Risk	
rs130058	*HTR1B*	2	0.000236	near Gene-5	A to T	T	NA	NA	NA	6	Enzyme	Risk	
rs615470	*CHRNA5*	2	0.000236	Intron	C to T	T	NA	NA	NA	15	Receptor	Protective	
rs7694646	*ADH4*	3	0.000265	Intron	A to T	A	NA	NA	NA	4	Enzyme	NA	2, 3

[a]For references, see the Supporting Information. [b]Effect that has the minor allele on the vulnerability to alcohol-related phenotypes. SNPs labeled with Protective/Risk have shown contrary results in different studies regarding their effect. [c]Linkage disequilibrium (LD) bin: 1: rs689-rs1229984-rs3762894; 2: rs1042364-rs1800759-rs7694646; 3: rs1042364-rs10893366-rs7694646; 4: rs1083366-rs10893365; 5: rs750338-rs3762894. [d]rs1800497 is considered a marker of both, *ANKK1* and *DRD2*. [e]C is the minor allele in Caucasians, whereas T is the minor allele in Asians. [f]In soluble form (*S-COMT*) or in membrane-bound form (*MB-COMT*) of the enzyme. A = adenine; AA = amino acid; Arg = arginine; Asn = asparagine; Asp = aspartic acid; C = cytosine; Chr. = chromosome; G = guanine; Gln = glutamine; Glu = glutamic acid; Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = metionine; mRNA = messenger ribonucleic acid; NA = not applicable; Pro = proline; SNP = single nucleotide polymorphism; T = thymine; Val = valine.

1992; Quertemont 2004; Edenberg 2007; Kimura *et al.* 2011). In Europeans and Africans, the frequency of the *ADH1B*2* allele is very low (approximately 5–10 percent) but its protective effect remains. As shown in a recent study by Bierut *et al.* (2012), the *ADH1B*2* allele is strongly associated with reduced risk for alcohol dependence in European American adolescents and young adults. The protective effect of the *ADH1B**2 allele on the risk for alcoholism relates to induced changes in the encoded amino acid chain. The presence of the A nucleotide in *ADH1B*2* allele carrier changes the amino acid residue 48 from arginine (Arg) to histidine (His). This amino acid substitution affects the enzyme binding site for the co-enzyme nicotinamide adenine dinucleotide (NAD+), which is required for alcohol catalysis (Peng & Yin 2009). The result is that the ADH enzyme will release the co-enzyme NAD+ more rapidly at the end of the reaction, increasing up to 80-fold the alcohol oxidization rate in comparison to the presence of the *ADH1B*1* allele (Edenberg 2007). This higher alcohol metabolism rate leads to an increase in acetaldehyde levels in the body, causing several aversive symptoms that would deter individuals from drinking alcohol. Although this hypothesis was successfully proven in animal studies (Rivera-Meza *et al.* 2010), humans with the *ADH1B*2* allele do not show accumulation of acetaldehyde after alcohol consumption, despite faster alcohol elimination (Mizoi *et al.* 1994; Neumark *et al.* 2004; Peng & Yin 2009), leading some authors to suggest that the increased generation of aldehyde can be effectively metabolized by hepatic and extrahepatic ALDHs (Peng & Yin 2009). The exact mechanism by which the *ADH1B*2* allele affects alcohol metabolism in humans is unknown, but the results of several studies indicate that the *ADH1B*2* allele significantly reduces the risk for alcoholism (Li, Zhao & Gelernter 2011).

The most significant SNP associated with alcohol-related phenotypes in this study was rs671, which belongs to the *ALDH2* gene. This gene encodes the mitochondrial enzyme ALDH2, which is expressed mainly in the liver and stomach, although it can also be found in other tissues, such as the brain (Yoshida *et al.* 1998). The functional SNP rs671 produces two different alleles of the gene: the allele *ALDH2*1*, which presents a glutamic acid at position 504 in the protein amino acid chain, and the allele *ALDH2*2*, in which the glutamic acid is substituted by the amino acid lysine. This second allele is much more frequent in the East Asian population (up to 30 percent) and almost absent in Caucasian or African population (Oota *et al.* 2004). The major distinction between the alleles lies in the catalytic activity of the encoded enzymes. The enzyme expressed by the *ALDH2*2* allele nearly completely lacks acetaldehyde metabolism activity, causing a prolonged and large accumulation of acetaldehyde in the blood after alcohol consumption. As a consequence, even a small amount of alcohol may produce high concentrations of acetaldehyde and causes several aversive symptoms, such as facial flushing, nausea, headache and tachycardia. This prevents a carrier of the *ALDH2*2* allele from excessive alcohol consumption and provides a significant reduction in risk for alcoholism (Yoshida *et al.* 1998).

On the contrary, variants in the *ADH1C* and *ADH4* genes have also been related to alcohol-dependence phenotypes. The most significantly associated *ALH1C* SNPs were the proxies rs698 and rs169342. Whereas the wild-type *ADH1C*1* includes the amino acids arginine at protein position 272 and isoleucine at position 350; in the more common *ADH1C*2* allele (rs698), the amino acid arginine changes to glycine; and in the rs169342, the isoleucine has changed to valine (Osier *et al.* 2002). As with *ADH1B*2*, the *ADH1C*1* encoded enzyme is highly active at oxidizing alcohol. In fact, in the presence of the *ADH1C*1* allele in homozygosis, the encoded enzyme possesses a 70 percent higher turnover rate and a 2.5-fold higher maximal velocity than enzymes encoded by the *ADH1C*2* allele in homozygosis (Edenberg 2007; Latella *et al.* 2012). That means that *ADH1C*1* reduces the predisposition to alcoholism in the same manner as *ADH1B*2*.

Regarding *ADH4*, the SNP with the lowest *P*-value in this study was rs1800759 (combined $P = 9.38 \times 10^{-7}$). This SNP is a functional mutation at the promoter region of *ADH4*, consisting of a nucleotide change from A to C, with the A allele showing a twofold higher promoter activity in transfected cells compared with the C allele (Edenberg, Jerome & Li 1999). Later studies linked this SNP to alcohol dependence in European- and African-American (Luo *et al.* 2005, 2006), Caucasian (Macgregor *et al.* 2009), European- and African-Brazilian (Guindalini *et al.* 2005), and German-Polish populations (Preuss *et al.* 2011). The authors suggested that higher expression of the enzyme in the presence of the A allele may be protective against alcohol dependence, in the same way that higher activity variants of *ADH1B* and *ADH1C* are the protective alleles for alcohol addiction phenotypes (Guindalini *et al.* 2005; Edenberg 2012). Other SNPs in the *ADH4* gene highly significantly associated with alcohol-related phenotypes are rs7694646, its proxies SNP rs104236 and rs3762894. Rs104236 is a non-synonymous but apparently non-functional SNP at exon 9 of *ADH4* whose major allele, G, has repeatedly been shown to be significantly associated with a risk of alcohol dependence (Luo *et al.* 2005; Edenberg *et al.* 2006; Preuss *et al.* 2011; Turchi *et al.* 2012). However, to the best of our knowledge, no study has been published regarding the biological explanation for this association. The rs3762894 variant at the 5′ end

of *ADH4* presumably affects the kinetic property of ADH4 so that the isoenzyme produced by the minor allele is more active than that encoded by the major allele. This may result in faster formation of acetaldehyde and therefore a stronger physiological reaction to alcohol, preventing C carriers of consuming elevated quantities of alcohol (Thomasson, Beard & Li 1995; Gizer *et al.* 2011).

The pairwise LD analysis has shown that rs3762894 is in high LD with the *ADH1B*2* (rs1229984) and *ADH1C*1* (rs698) SNPs mentioned earlier (Fig. 2). These SNPs form an LD group of protective SNPs against alcohol addiction apparently by elevating levels of acetaldehyde in the body by increasing its formation or reducing its degradation. Nevertheless, as stated by some authors, it is also possible that only *ADH1B*2* has a true impact on alcohol addiction and that the other SNPs show only association with alcohol phenotypes because of this high LD (Osier *et al.* 1999; Borràs *et al.* 2000; Choi *et al.* 2005). However, and despite the controversial nature of these results, a recent meta-analysis conducted by Li *et al.* (2012) indicates that at least *ADH1C*1* independently reduces the risk for alcoholism. Although the frequency for the *ADH1C*1* allele is higher in Asians than in other populations, it is much more frequent in European populations than *ADH1B*2* and can be present in up to 50 percent of individuals (for a review, see Li *et al.* 2012).

*Variants in the ankyrin repeat and kinase domain containing 1 (*ANKK1*)-dopamine receptor 2 (*DRD2*) genes*

Although *ADH1B* and *ALDH2* genes harbor the most replicated SNPs associated with alcohol-related phenotypes, the dopaminergic system is the most explored in relation to substance dependence and addictive behaviors as well as other various neuropsychiatric disorders, especially those in association with the dopaminergic D2 receptor (Noble 2003).

The rs1800497, commonly called Taq1A, was the first SNP related to the *DRD2* gene to be associated with severe alcoholism (Blum *et al.* 1990). Since then, Taq1A is the variant that has received the most attention in molecular studies related to psychiatric disorders (Ponce *et al.* 2009). Nevertheless, in 2004, Neville and colleagues showed that Taq1A does not belong to *DRD2* but to a nearby gene called repeat and kinase domain-containing 1 (*ANKK1*), a member of a serine/threonine kinase protein family involved in signal transduction pathways (Neville, Johnstone & Walton 2004). More recent studies discovered the cellular localization of the *ANKK1* gene, and evidence showed that this gene is expressed exclusively in astrocytes in human, rat and mouse (Hoenicka *et al.* 2010). Astrocytes are implicated in learning processes of addictive behaviors and also serve an important role in the rewarding effects of drugs. This could justify a role for *ANKK1* and Taq1A in

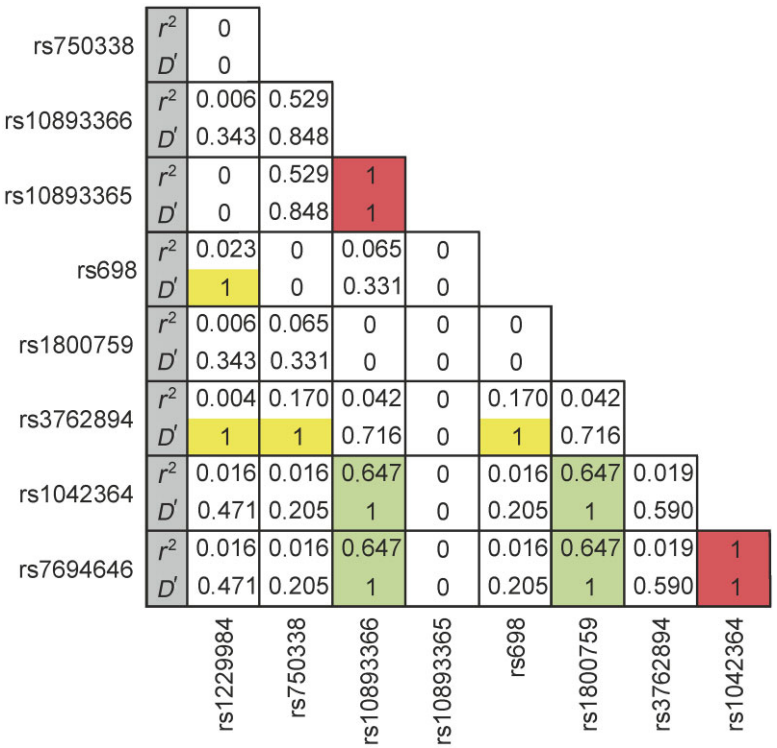

Figure 2 Pairwise linkage disequilibrium (LD) plot of single nucleotide polymorphism (SNP) significantly associated with alcohol-related phenotypes. SNPs rs1800497 (*DRD2/ANKK1*), rs16139 (*NYP*), rs279858 (*GABRA2*), rs179971 (*OPRM1*), rs701492 (*GAD1*), rs4680 (*COMT*), rs16944 (*IL1B*), rs130058 (*HTR1B*) and rs615470 (*CHRNA5*) were excluded from this plot because their retrieved r^2 and D' values of 0 with all other SNPs. Red squares: high LD (r^2: 0.8–1; D': 0.9–1). Green squares: medium LD (r^2: 0.6–0.79; D': 0.8–0.9). Yellow squares: low r^2 values (<0.6) but $D'=1$

drug-related phenotypes (Miguel-Hidalgo 2009). In this study, we found that Taq1A is the third most significant SNP associated with alcohol-related phenotypes, with a combined *P*-value of 3.20×10^{-12}, including 12 independent studies (Table 2).

Taq1A consists of a cytosine to thymine nucleotide change in exon 8 of the *ANKK1* gene. In the literature, these two alleles are commonly referred to as A2 (C nucleotide) and A1 (T nucleotide) and cause a glutamic acid (A2 allele) to lysine (A1 allele) amino acid substitution at position 713 in the ANKK1 protein (Neville *et al.* 2004). The functional implication of this amino acid alteration has not been completely determined, although it has been associated with several phenotypes. In a similar fashion, the presence of the A1 allele (A1/A1, A1/A2 genotype) was related to lower relative glucose rate in the brain; reduced amplitude and prolonged latency of the event-related potential P300; lower grow hormone release; and, in alcoholics, an elevated dopamine active transporter density compared with homozygous carriers of the A2 allele (Blum & Noble 1994; Gabby *et al.* 1996; Ritchie & Noble 1996; Noble *et al.* 1997; Hill *et al.* 1998; Eriksson *et al.* 2000; Laine *et al.* 2001; Ratsma *et al.* 2001).

However, what may be the most important and controversial phenotypical association is the one linking the A1 allele to a significant reduction in D2 receptor density in the striatum by 30–40 percent in comparison to the A2/A2 genotype (Noble *et al.* 1991; Thompson *et al.* 1997; Pohjalainen *et al.* 1998; Jönsson *et al.* 1999). In addition, Laakso *et al.* (2005) found that the A1 allele is associated with increased striatal activity of the aromatic L-amino acid decarboxylase enzyme, the final enzyme in the dopamine biosynthesis (Laakso *et al.* 2005). As suggested by the authors, the lower D2 receptor expression induced by the presence of the A1 allele would cause decreased autoreceptor functioning and, as a consequence, increased synthesis of dopamine. These findings indicate a functional relationship between the *DRD2* and *ANKK1* genes. This is plausible because both genes are located in the same gene cluster, overlap and share haplotypic blocks, and their promoters have identical *cis* elements (i.e. regions of DNA that regulate gene expression) (Neville *et al.* 2004; Hill *et al.* 2008; Hoenicka *et al.* 2010). In addition, a recent *in vitro* study carried out by Hoenicka *et al.* (2010) showed that *Ankk1* expression in mouse astrocytes increased in response to the dopamine receptor agonist apomorphine. Therefore, Taq1A may influence *DRD2* expression and dopamine synthesis by altering the normal functioning of ANKK1 (Laakso *et al.* 2005).

Taq1A is in high LD with different *DRD2* polymorphisms and a number studies have suggested that some phenotypes previously associated with Taq1A may actually be driven by this *DRD2* polymorphism. This is especially true with C957T (rs6277), a synonymous SNP in exon 7 of the *DRD2* gene that alters mRNA folding and decreases *DRD2* mRNA stability, receptor synthesis and binding potential *in vitro* and *in vivo* (Duan *et al.* 2003; Hirvonen *et al.* 2004). Hirvonen *et al.* (2004) concluded that the former relationship found between Taq1A and the reduction in striatal D2 receptor density was, in fact, due to its high association with C957T. However, the data from Laakso *et al.* (2005) clearly showed that the increase of aromatic L-amino acid decarboxylase activity during the dopamine biosynthesis was associated with Taq1A and not C957T or other polymorphisms. In 2008, Ponce and colleagues carried out an interaction study between C957T and Taq1A in a group of Spanish alcohol-dependent patients with psychopathic traits (Ponce *et al.* 2008). The results showed that the risk genotypes for both SNPs, CC for C957T and A1 allele for Taq1A, were significantly overrepresented in alcohol-dependent patients with co-morbid dissocial personality disorder, leading the authors to suggest an epistatic relationship between the Taq1A and C957T SNP and an interaction between *ANKK1* and *DRD2* in neurotransmitter pathways, affecting the individual's vulnerability to drug addiction and psychopathic traits.

To date, more and more evidence have arisen, which suggest that Taq1A is a marker of a genetic variant of both *ANKK1* and *DRD2* genes. Similarly, it is also associated with other SNPs in the *DRD2* gene aside from the already mentioned relationship between the Taq1A and C957T SNPs (i.e. the intronic SNPs rs2283265 and rs1076560) that affect mRNA expression, splicing and neuronal activity in working memory pathways (Zhang *et al.* 2007).

*Variants in the mu-Opioid receptor (*OPRM1*) gene*

Several drugs of abuse, such as alcohol, nicotine, cannabis and cocaine, exert their rewarding effects by increasing dopamine activity in the cortico-mesolimbic system. The endogenous opioid system is implicated in the modulation of these effects and suffers significant adaptive changes in response to drug exposure. Therefore, the endogenous opioid system plays an important role in the development of drug addiction (for a review, see Trigo *et al.* 2010). Particularly for alcohol addiction, evidence implicating the mu-opioid receptor (MOR) are driven from two main facts: first, that inactivation of MOR in the ventral tegmental area (VTA) inhibits alcohol-induced dopamine release and, second, the effectiveness of the opioid antagonist naltrexone in the treatment of alcohol dependence (Heilig *et al.* 2011; Hillemacher *et al.* 2011).

The A118G SNP (rs1799971) is one of the most prevalent functional variants in the *OPRM1* gene and has

the highest overall frequency of any reported *OPRM1* coding variant (Bond *et al.* 1998; Kroslak *et al.* 2007). This SNP is a functional A to G nucleotide substitution at position 118 of the gene that encodes an asparagine to aspartate change at position 40 at exon 1 (Asn40Asp) in the N-terminal region of the protein chain. This amino acid change decreases the number of N-linked glycosylation sites from five to four in the MOR (Shabalina *et al.* 2009). This N-glycosylation has been reported to be necessary for correct sorting of receptors to plasma membrane, ligand-binding affinity and signal transduction, among other functional implications (Kroslak *et al.* 2007).

In one of the first *in vitro* studies on the functional relevance of this SNP, Bond *et al.* (1998) reported that the presence of the G allele increases the affinity of the receptor for β-endorphin by threefold in addition to increasing the power of beta-endorphin to activate G protein-coupled potassium channels, resulting in alteration of the signal transduction pathways. Years later, Zhang *et al.* (2005) reported that mRNA transcript levels were reduced by twofold with the G allele in comparison to A allele in human post-mortem brains along with 10-fold lower protein expression in stable transfected cell lines. Also, lower surface receptor expression with the G allele was observed by Kroslak *et al.* (2007) as well as decreased AMP activation and lower agonist-induced MOR activation. However, several studies were not able to reproduce these findings, and the molecular mechanism underlying functional implications of the A118G polymorphisms remains unclear (Oertel *et al.* 2009). Nevertheless, there is important evidence concerning the effect of the A118G variance on the clinical treatment of alcohol-dependent patients with naltrexone. Several studies have shown that G carriers had a significantly better clinical outcome with the opioid antagonist than A carriers (Oslin *et al.* 2003; Ray & Hutchison 2004, 2007), making the A118G an important marker to take into account in the pharmacological treatment of alcohol dependence.

*Variants in the catecol-O-metiltransferase (*COMT*) gene*

As previously mentioned, the dopaminergic system has been frequently associated with substance dependence and psychiatric disease. The COMT protein is an Mg^{2+}-dependent enzyme responsible for degrading catecholamine neurotransmitters such as dopamine, adrenaline and noradrenaline. The implication of the COMT enzyme in the metabolism of dopamine makes the *COMT* a suitable candidate gene for the mechanism of alcohol addiction (Voisey *et al.* 2011). The *COMT* gene is located on chromosome 22q11 and encodes two different isoforms of the enzyme: one long membrane-binding isoform (*MB-COMT*) and one short soluble isoform (*S-COMT*) (Chen *et al.* 2004). In the brain, the existing COMT enzymes are almost exclusively *MB-COMT* isoform, whereas the *S-COMT* is predominantly expressed in tissues such as the liver, blood and kidney (Tenhunen *et al.* 1993; Matsumoto *et al.* 2003). The most frequently studied variant within the *COMT* gene is a functional SNP located in exon 4, which consists of a substitution of the amino acid valine (Val) to methionine (Met). On the *MB-COMT*, the substitution takes place at protein position 158, whereas on the *S-COMT*, it is located at position 108 (Lachman *et al.* 1996). The Val158/108Met polymorphism has been shown to affect the thermostability of the enzyme, leading to modification of its enzymatic activity. The Val high-activity allele has a four times greater catabolic rate than the lower active Met allele. This higher degradation rate leads to a decrease of dopamine levels, especially in the prefrontal cortex and in the hippocampus (Chen *et al.* 2004; Honea *et al.* 2009; Dennis *et al.* 2010), which eventually may lead to inefficient frontal lobe function, affecting cognitive processes such as working memory and executive functioning, among others (for a review, see Dickinson & Elvevåg 2009). The less active Met allele, despite the likely better frontal lobe function, has been linked to negative emotional states such as increased anxiety and decreased stress resilience (Ducci & Goldman 2008). In this way, the Val158/108Met SNP has been associated with various affective and personality traits such as impulsivity, extraversion, novelty seeking and reward dependence/sensation seeking (Dickinson & Elvevåg 2009), all of which are, in turn, related to drug addiction.

One of the first studies relating the Val158/108Met polymorphism to alcohol dependence phenotypes was conducted by Tiihonen *et al.* (1999), who associated the low active Met allele with alcoholism, and followed with studies that confirmed this association (i.e. Wang *et al.* 2001). The authors showed that the Met allele carrier had lower dopamine inactivation and suggested that this would explain the increased vulnerability to alcohol addiction because alcohol-induced euphoria has been frequently associated with rapid release of dopamine in the limbic areas (Tiihonen *et al.* 1999; Morgan & Badawy 2001; Voisey *et al.* 2011). On the contrary, other studies showed that the highly active Val allele was associated with alcohol dependence (Enoch *et al.* 2006; Serý *et al.* 2006). The authors of the latter study stated that this opposite finding might be explained by gender differences in the sample, co-dependence with other drugs and the influence of gene–environment interactions (Enoch *et al.* 2006).

*Variants in the gamma-aminobutyric acid A receptor α-2 (*GABRA2*) gene and the glutamate decarboxylase (*GAD1*) gene of the GABAergic system*

The $GABA_A$ receptor has been implicated in the pharmacological actions of alcohol, including tolerance, withdrawal, dependence, sedation and anxiolysis, as well as cross-tolerance to other drugs such as benzodiazepines and barbiturates (Ducci & Goldman 2008; Haughey *et al.* 2008a). Data indicate that alcohol modulates the GABA receptor complex in an allosteric way, hyperpolarizing cells by opening the chloride channel (Koob 2004).

One of the first association studies linking *GABRA2* to alcohol dependence was carried out by Edenberg *et al.* (2004). After examining 69 SNPs in the $GABA_A$ receptor subunits, they found a significant association between more than 30 SNPs in the *GABRA2* subunit and alcohol dependence, including a 3-SNP haplotype (composed of rs279871, rs279845 and rs279836) associated with the phenotype at a *P*-value of 2×10^{-8}. In the same year, Covault *et al.* (2004) also found an association between alcohol dependence and several *GABRA2* SNPs localized in the same gene region as the SNPs identified by Edenberg *et al.* (2004) in a European-American population. Finally, Ittiwut *et al.* (2012) replicated the results of Covault *et al.* (2004) in a population of African-Americans by finding significant associations between a 3-SNP risk haplotype in *GABRA2* (all included also in Covault *et al.*'s study) and alcohol dependence. Nevertheless, the biological pathway by which this subunit interacts with alcohol remains unclear.

One SNP in this *GABRA2* subunit, rs279858, was highly significantly associated with alcohol-related phenotypes (combined $P = 2.88 \times 10^{-8}$). This SNP leads to a synonymous A to G nucleotide substitution at position 132 in exon 5 of the gene and was also associated with alcohol dependence by Edenberg *et al.* (2004) and Covault *et al.* (2004). In the last few years, this SNP was associated with alterations in the mRNA level of *GABRA2* in the prefrontal cortex (Haughey *et al.* 2008b), meaning that in the AA genotype, the mRNA level was twofold higher than the AG genotype. However, no differences in protein expression were found, and no difference was observed between alcoholics and non-alcoholics. The authors speculated that the relationship between *GABRA2* and alcohol dependence could be a result of alterations in the receptor function caused by changes in gene transcription and/or translation, although the small sample size used in the study did not allow for confidently demonstrating alteration in gene transcription.

In addition to the GABA receptor, GAD1, the enzyme responsible for synthesizing and maintaining the basal level of GABA in the brain, has been studied for its implication in alcohol dependence. Two independent studies showed a significant association between the intronic SNP rs701492 of the *GAD1* gene (combined *P*-value of 1.75×10^{-5}) and alcohol consumption in Caucasians and Han Chinese (Loh *et al.* 2006; Tabakoff *et al.* 2009, respectively). It is known that individuals with deficient GAD1 functioning are more likely to develop anxiety disorders and are therefore at higher risk for developing alcohol dependence. Although the specific role of rs701492 is not known, it is therefore possible that it may affect GAD1 function and increase vulnerability to alcohol consumption.

*Variants in the serotonin receptor 1B (*HTR1B*) gene*

The serotonergic system presents dense projections in several brain circuits and areas highly implicated in reward mechanisms. Many studies have associated serotonin (5-HT) receptors with alcoholism, namely the $5\text{-}HT_{1B}$ receptor. This serotonin receptor subtype is expressed in brain regions that modulate the rewarding effects of alcohol (such as the ventral tegmental area and nucleus accumbens) (Hu *et al.* 2010; Furay *et al.* 2011) and evidence shows that $5\text{-}HT_{1B}$ receptors increase dopaminergic tone in the mesolimbic reward pathway (Furay *et al.* 2011). Studies have shown that $5\text{-}HT_{1B}$ knockout mice exhibit greater aggression and impulsivity behaviors as well as increased alcohol consumption (Saudou *et al.* 1994; Crabbe *et al.* 1996; Bouwknecht *et al.* 2001). Furthermore, pre-clinical pharmacological studies have shown that $5\text{-}HT_{1B}$ agonists reduce alcohol drinking and alcohol-induced aggression and impulsiveness (Cao *et al.* 2011).

The SNP rs13008 (also called A-161T) lies in the 5′ regulatory region of the $5\text{-}HT_{1B}$ gene and represents a nucleotide change from A to T at position 161 (Sun *et al.* 2002; Lee *et al.* 2009; Cao *et al.* 2011). This variant has been associated with alcohol dependence in Taiwanese and Chinese Han population (Sun *et al.* 2002; Cao *et al.* 2011), but association results were negative in studies using Caucasian, African and Hispanic-American populations (Cao *et al.* 2011). Although, to date, there has been no direct biological evidence to explain the association between this gene variant and alcohol addiction phenotypes, the study conducted by Sun *et al.* (2002) showed that the A-161T variants may affect $5\text{-}HT_{1B}$ gene expression in a tissue-specific manner.

Other highly significant SNPs associated with alcohol-related phenotypes

There are other SNPs that are highly significantly associated with alcohol-related phenotypes, but the results have been replicated only once or twice. These SNPs represent therefore valuable candidate gene markers for further addiction genetics research. This is the case for

the *GAD1* SNP rs701492 mentioned earlier, as well as for the rs16139 in the Neuropeptide Y (*NPY*) gene. This SNP is implicated in intracellular processing of the pre-protein of NPY and release of the mature NPY. Heterozygous carriers (Pro7/Leu7) have been shown to possess 47 percent more NPY plasma concentration than homozygous Leu7 carriers (Lappalainen *et al.* 2002). In the two studies included in this review, results regarding the risk allele Pro7 were contrary, as it was identified as a protective allele by Kovanen *et al.* (2010) and a risk allele by Lappalainen *et al.* (2002).

Another promising candidate gene for addiction genetics research is *PKNOX2*. Three SNPs in this gene were in the top 20 list of most significant SNPs associated with alcohol-related phenotypes. The protein encoded by *PKNOX2* is a nuclear transcription factor, which, in initial pre-clinical studies with mice, was implicated in alcohol addiction (Mulligan *et al.* 2006). A later study with humans has associated eight SNPs in *PKNOX2*, including the three SNPs included in this study, with substance abuse in European women, including lifetime alcohol, nicotine, marijuana, cocaine and other drug dependencies (Chen *et al.* 2011). This association between *PKNOX2* and alcohol dependence, especially with the SNP rs750338, has been replicated by two other studies (Bierut *et al.* 2010; Wang *et al.* 2011). Further studies should confirm these associations and explore the involvement of *PKNOX2* in common pathways underlying addiction to different drugs of abuse. Similarly, an SNP in *CHRNA5*, rs615470, has been associated with alcohol-related phenotypes in two independent studies (combined *P*-value = 0.00024). Because this gene is highly implicated in nicotine addiction (see the section Variants in *nAChR* genes), and nicotine and alcohol are both frequently co-abused, it is plausible that this gene is related to common biological pathways between both types of addiction. The issue of common pathways underlying different drug dependencies will be further discussed in the section Common SNPs underlying drug addiction: nicotinic receptors and the endocannabinoid system.

Finally, a variation in the interleukin-1 beta (*IL1β*) gene has been significantly associated with alcohol abuse. IL1β is a cytokine protein that plays a crucial role in the immune and inflammatory responses. Alcohol enhances actions of the immune system by causing glial cells to release cytokines (such as IL1β), and studies with rats have shown that chronic ethanol intake leads to increased IL1β levels in the cerebral cortex (Vallés *et al.* 2004). This release of IL1β can increase *N*-methyl-D-aspartate receptor activity and dopamine release in the nucleus accumbens, increasing drug reward and dependence (Liu *et al.* 2009). The SNP rs16944 (also known as IL1β-511) has been shown to affect IL1β expression (di Giovine *et al.* 1992) and increase protein secretion in the blood by two- to threefold (Hall *et al.* 2004). Nevertheless, results on the functional aspect of this SNP require further investigation.

SNP and NICOTINE-related phenotypes

As opposed to alcohol-related phenotypes, few candidate gene and GWAS have been published on nicotine-related phenotypes. One hundred twenty-three publications regarding nicotine-related phenotypes have been included in this study. Altogether, these publications retrieved 435 SNPs tagging 124 different genes and 62 non-genic SNPs. Table 3 shows the 20 most significant SNPs associated with nicotine-related phenotypes. The vast majority of these SNPs belong to nicotinic acetylcholine receptor (*nAChR*) genes, mainly to the *CHRNA5-CHRNA3-CHRNB4* gene cluster and neighboring genes such as the iron-responsive element binding protein (IREB2).

Variants in nAChR *genes*

Nicotine exerts its physiological effects through binding to nAChRs. This makes the AChR-encoding genes perfect candidates for influencing smoking and nicotine dependence. There exist 16 different nicotinic receptor subunit genes that encode a set of different nicotinic receptor subunit proteins (for a detailed review on nAChRs, see Dani & Bertrand 2007; Albuquerque *et al.* 2009). In the last few years, most of the research on addiction genetics has focused on genes encoding the α5, α3 and β4 units. These genes, *CHRNA5*, *CHRNA3* and *CHRNB4*, are organized in a gene cluster on chromosome 15q24; several gene variants in this locus have been associated with smoking behavior and nicotine dependence, as well as smoking-related diseases such as lung cancer and chronic obstructive pulmonary disease (Bierut 2010; Saccone *et al.* 2010; Wassenaar *et al.* 2011). As shown in Table 3, only 6 SNPs of the top 20 most highly associated with nicotine-related phenotypes do not belong directly to the CHRN gene family, but 4 are located in the neighboring genes. Performing pairwise LD analysis of these genes retrieves strong LD patterns between nearly all of these SNPs (Fig. 3a), especially a group of 14 SNPs shown to be in extremely high LD with each other (Fig. 3b). The minor alleles of most of these SNPs significantly increase risk for nicotine dependence and smoking behavior phenotypes. This observation is in line with a study where the authors explored the association between several nicotine dependence phenotypes and *CHRN* haplotypes, including rs16969968 and rs1051730. Haplotypes where the minor alleles of these SNPs were present were significantly associated with

Table 3 Most significantly associated SNPs with nicotine-related phenotypes.

SNP	*Gene*	*No. of studies*[a]	*Combined* P-*value*	*SNP function*	*Allele change*	*Minor allele*	*mRNA position*	*AA change*	*Protein position*	*Chr.*	*Protein function*	*Effect*[b]	*LD bin*[c]
rs1051730	*CHRNA3*	21	5.29×10^{-47}	Synon, Exon 5	G to T	T	1146	Try to Try	215	15	Receptor	Risk	1
rs16969968	*CHRNA5*	17	1.16×10^{-31}	Non-syn, Exon 6	G to A	A	1392	Asp to Asn	398	15	Receptor	Risk	1
rs2036527	*CHRNA5*	3	4.47×10^{-16}	Near gene initiation sequence	C to T	T	NA	NA	NA	15	Receptor	Risk	1.2
rs8034191	*LOC123688/HYKK*	4	2.52×10^{-12}	Intron	C to T	C	NA	NA	NA	15	Enzyme	Risk	1
rs637137	*CHRNA5*	3	3.43×10^{-12}	Intron	A to T	A	NA	NA	NA	15	Receptor	Protective	2
rs17487223	*CHRNB4*	4	5.28×10^{-12}	Intron	C to T	T	NA	NA	NA	15	Receptor	Risk	1
rs17483548	*IREB2*	3	1.58×10^{-11}	Near Gene-5	A to G	A	NA	NA	NA	15	Binding	Risk	1
rs951266	*CHRNA5*	3	1.22×10^{-10}	Intron	C to T	T	NA	NA	NA	15	Receptor	Risk	1
rs1800497	*ANKK1/DRD2*[d]	11	1.41×10^{-10}	Non-syn, Exon 8	C to T	T	2231	Glu to Lys	713	11	Enzyme/Receptor	Risk	
rs3743078	*CHRNA3*	4	1.12×10^{-09}	Intron	C to G	G	NA	NA	NA	15	Receptor	Protective	1[e]
rs684513	*CHRNA5*	3	1.26×10^{-09}	Intron	C to G	G	NA	NA	NA	15	Receptor	Protective	2
rs569207	*CHRNA5*	2	1.44×10^{-09}	Intron	A to G	A	NA	NA	NA	15	Receptor	Protective	2
rs17484235	*IREB2*	2	3.13×10^{-09}	Intron	C to G	G	NA	NA	NA	15	Binding	Risk	1
rs1317286	*CHRNA3*	4	6.18×10^{-09}	Intron	A to G	G	NA	NA	NA	15	Receptor	Risk	1
rs17486278	*CHRNA5*	5	1.06×10^{-08}	Intron	A to C	C	NA	NA	NA	15	Receptor	Risk	1
rs6474413	*CHRNB3*	4	2.39×10^{-08}	Near Gene-5	C to T	C	NA	NA	NA	15	Receptor	Protective/Risk	2
rs12440014	*CHRNB4*	2	2.95×10^{-08}	Intron	C to G	G	NA	NA	NA	15	Receptor	Protective/Risk	1
rs17483686	*IREB2*	2	3.61×10^{-08}	Intron	A to T	T	NA	NA	NA	15	Binding	Risk	1
rs3813567	*CHRNB4*	3	3.75×10^{-08}	Near Gene-5	C to T	C	NA	NA	NA	15	Receptor	Protective	1[e]
rs3762607	*GABRA4*	3	1.29×10^{-07}	Near Gene-5	A to G	G	NA	NA	NA	4	Receptor	Protective	2

[a]For references, see the Supporting Information. [b]Effect that has the minor allele on the vulnerability to nicotine-related phenotypes.SNPs labeled with Protective/Risk have shown contrary results in different studies regarding their effect. [c]Linkage disequilibrium (LD) bin: 1: rs1051730-rs16969968-rs2036527-rs8034191-rs17487223-rs17483548-rs951266-rss3743078-rs17484235-rs1317286-rs17486278-rs12440014-rs17483686-3813567; 2: rs2036527-rs637137-rs684513-rs569207-rs6474413-rs3762607. [d]rs1800497 is considered a marker of both, *ANKK1* and *DRD2*. [e]rs3743078 and rs3813567 are NOT in LD with each other. A = adenine; AA = amino acid; Asn = asparagine; Asp = aspartic acid; C = cytosine; Chr. = chromosome; G = guanine; Glu = glutamic acid; Lys; lysine; mRNA = messenger ribonucleic acid; NA = not applicable; SNP = single nucleotide polymorphism; T = thymine; Try = tryptophan.

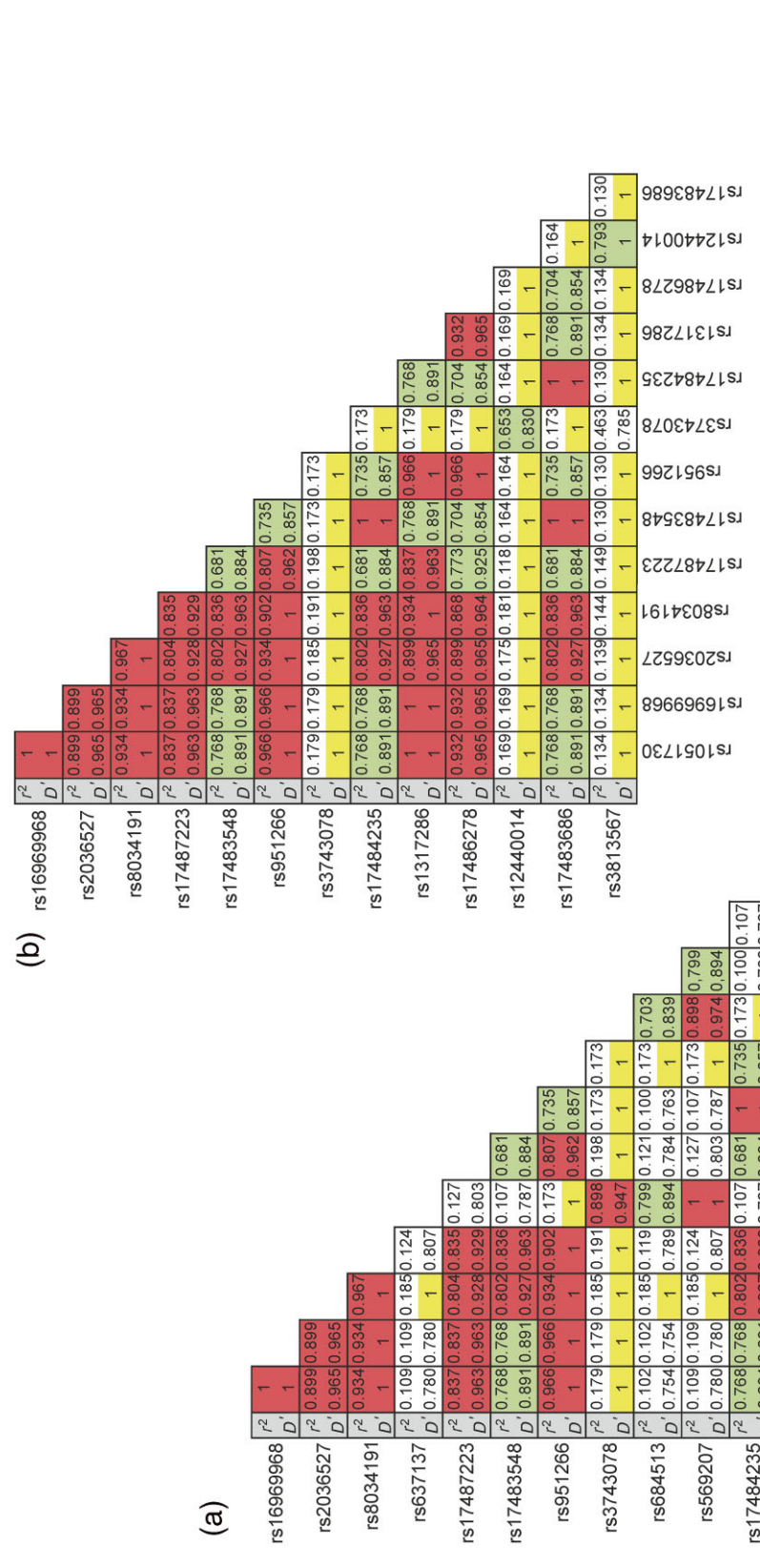

Figure 3 Pairwise linkage disequilibrium (LD) plot of the most significantly associated single nucleotide polymorphisms (SNPs) with nicotine-related phenotypes. (a) Pairwise LD values for all selected highly significantly associated SNPs. SNPs rs1800497 (*ANKK1*/*DRD2*), rs6474413 (*CHRNB3*) and rs3762607 (*GABRA4*) were excluded from this plot because their retrieved r^2 and D' values of 0 with all other SNPs. (b) Subgroup of 14 SNPs in high LD. Excluding three SNPs of plot (a), all SNPs are in medium to high LD with each other. Red squares: high LD (r^2: 0.8–1; D': 0.9–1). Green squares: medium LD (r^2: 0.6–0.79; D': 0.8–0.9). Yellow squares: low r^2 values (<0.6) but D' = 1

increased risk for heavy, pervasive and automatic smoking, as well as with less severe withdrawal syndromes (Baker *et al.* 2009).

The most interesting and popular of these 14 highly significant 'risk' SNPs is the rs16969968 SNP in the *CHRNA5* gene because it is the only one with a non-synonymous amino acid change in the protein. This missense polymorphism changes the amino acid aspartate (Asp) to asparagine (Asn) at position 398 in the cytoplasmic loop between transmembrane domains. The Asn allele is the risk allele. While carriers of one copy of the Asn allele increased the risk of becoming nicotine dependent by 1.3-fold, in homozygous carriers, the risk increased up to twofold compared with the homozygous Asp genotype carrier (Bierut 2010). The frequency of the risk allele is very heterogeneous across different populations, being relatively common in European and Middle Eastern people (37–43 percent) but very rare or inexistent in Africans, East Asians and Native Americans (Bierut *et al.* 2008). The underlying biological functional implications of the rs16969968 polymorphism have been the focus of investigation since its study began. The first *in vitro* studies showed that in the presence of the Asn risk allele, the receptor response to agonists (i.e. epibatidine) is significantly reduced (by twofold) in comparison to the presence of the Asp allele (Bierut *et al.* 2008). Because no differences in gene expression have been found between the two alleles, the variants might affect receptor functioning without affecting receptor expression. Nevertheless, *CHRNA5* mRNA levels are partially affected by different *cis*-acting polymorphisms (Wang *et al.* 2009). One study analyzed the presence of the rs16969968 variant on the background of this *cis*-polymorphism-induced *CHRNA5* mRNA variation in the frontal cortex and found occurrence of the Asn allele more frequently in the presence of low mRNA expression of *CHRNA5* (Wang *et al.* 2009). In addition, pre-clinical studies with α5 knockout mice, which emulate inactivation of the receptor protein due to the presence of the rs16969968 Asn allele, suggest that loss of α5 functioning reduces sensitivity to adverse effects of nicotine (Jackson *et al.* 2010). These results were corroborated by Fowler *et al.* (2011) by showing that the α5 knockout mice consumed a greater amount of nicotine at increasing dosages and a greater motivation to seek and obtain nicotine at higher doses than wild-type mice.

Rs16969968 shows perfect and complete LD with the *CHRNA3* SNPs rs1051730 and rs1317286. These three SNPs are in complete and perfect LD ($D' = 1$, $r^2 = 1$). The rs1051730 is a synonymous SNP at exon 5 of the *CHRNA3* gene and is the most significantly associated SNP with nicotine phenotypes of all top 20 SNPs (combined $P = 5.29 \times 10^{-47}$). Nevertheless, it is commonly accepted that the biological significance of the association between this SNP and nicotine addiction and smoking behavior is most likely attributable to rs16969968.

Interestingly, aside from the 14 SNP LD group, the LD analysis indicated a small second subgroup of SNPs in high LD, including SNPs not belonging to the first group, whose minor alleles have a protective effect on vulnerability to nicotine addiction and smoking behavior. This group included the three *CHRNA5* SNPs rs569207, rs637137 and rs684513 and the *CHRNA3* SNP rs3743078, which was also present in the 14 SNP risk group and was, in fact, one of the few SNPs showing a protective effect. All of the 'protective' SNPs lie in the intronic regions of *CHRNA5* and *CHRNA3*, and to date, it is not known how they may affect protein configuration or functioning to influence nicotine dependence and smoking.

Only one of the SNPs, belonging to the *CHRN* family listed in Table 3, rs6474413, was not in LD with any of the other *CHRN* SNPs or neighboring genes, being therefore independently associated with nicotine-related phenotypes. This independent association relies on its independency with the *CHRNA5-CHRNA3-CHRNAB4* gene cluster because the SNP is located in the *CHRNB3* gene, which, together with *CHRNA6*, clusters in chromosome 8p11. The minor allele of this SNP has repetitively demonstrated significant association with nicotine dependence in different studies (Bierut *et al.* 2007; Saccone *et al.* 2007, 2009a,b, 2010); but nevertheless, as stated by the authors, this SNP is most likely significantly associated with other two SNPs in *CHRNA3* because of its high LD, namely rs4953 and rs4952, which are the only known coding SNPs in this gene (Saccone *et al.* 2007).

Variants in ANKK1 *and* DRD2 *genes*

In addition to alcohol, Taq1A was positively associated with nicotine dependence in many studies (11 in this review). Nevertheless, the association with nicotine dependence or smoking behavior is much more controversial and recent meta-analysis found none or only weak associations (Munafò *et al.* 2004, 2009; Gorwood *et al.* 2012).

Other highly significant SNPs associated with nicotine-related phenotypes

One SNP belonging to the GABRA4 subunit was present in the top 20 most significantly associated SNPs with nicotine-related phenotypes. The non-functional SNP rs3762607 lies in the upstream promoter region of *GABRA4*, consisting of an A to G nucleotide change. Its association with nicotine dependence was first identified

Table 4 Most significantly associated SNPs with cannabis-related phenotypes.

SNP	*Gene*	*No. of studies*[a]	*Combined P-value*	*SNP function*	*Allele change*	*Minor allele*	*mRNA position*	*AA change*	*Protein position*	*Chr.*	*Protein function*	*Effect*[b]
rs806380	*CNR1*	2	2.78×10^{-03}	Intron	A to G	G	NA	NA	NA	6	Receptor	Protective
rs324420	*FAAH*	3	3.97×10^{-02}	Non-syn, Exon 3	C to A	A	467	Pro to Thr	129	1	Enzyme	Protective

[a]For references, see the Supporting Information. [b]Effect that has the minor allele on the vulnerability to cocaine-related phenotypes. A = adenine; AA = amino acid; C = cytosine; Chr = chromosome; G = guanine; mRNA = messenger ribonucleic acid; NA = not applicable; Pro = proline; SNP = single nucleotide polymorphism; Thr = threonine.

Table 5 Most frequently associated SNPs with cocaine-related phenotypes.

SNP	*Gene*	*No. of studies*[a]	*Combined P-value*	*SNP function*	*Allele change*	*Minor allele*	*mRNA position*	*AA change*	*Protein position*	*Chr.*	*Protein function*	*Effect*[b]
rs16969968	*CHRNA5*	3	7.64×10^{-05}	Non-syn, Exon 6	G to A	A	1392	Asp to Asn	398	15	Receptor	Protective
rs806368	*CNR1*	2	6.49×10^{-03}	UTR-3, Exon 4	T to C	C	NA	NA	NA	6	Receptor	Protective

[a]For references, see the Supporting Information. [b]Effect that has the minor allele on the vulnerability to cocaine-related phenotypes. A = adenine; AA = amino acid; Asn = asparagine; Asp = aspartic acid; C = cytosine; Chr. = chromosome; G = guanine; mRNA = messenger ribonucleic acid; NA = not applicable; SNP = single nucleotide polymorphism; T = thymine.

in a study carried out by Saccone *et al.* (2007) and further explored later by Agrawal *et al.* (2008), showing the minor allele protective effects on vulnerability to nicotine dependence (Agrawal *et al.* 2008). More recently, a linkage scan study for different drugs of abuse (including nicotine, alcohol, cannabis and cocaine) retrieved significant associations with a region of chromosome 4 that includes *GABRA4*. Variants in the $GABA_A$ receptor have previously been highly associated with different drugs of abuse. Namely, the *GABRA2* subunit has repeatedly been linked to alcohol abuse and dependence [see the section Variants in the gamma-aminobutyric acid A receptor α-2 (*GABRA2*) gene and the glutamate decarboxylase (*GAD1*) gene of the GABAergic system]. To date, few studies have explored the association between *GABRA4* and addiction, but in the light of these results, it seems a promising candidate for future research.

SNP and CANNABIS- and COCAINE-related phenotypes

Unlike alcohol and nicotine, little research has been conducted on cannabis and cocaine addiction-associated genetic variants. Only two SNPs have been replicated once or twice in independent studies both for cannabis and cocaine (Tables 4 and 5, respectively). Interestingly, studies noted an association between SNPs of the endocannabinoid system for cannabis as well for cocaine, although the associated SNPs are different. For cocaine, the α5 receptor subunit has been implicated, as it was for alcohol and nicotine. Because of the little available information and the partially overlapping evidence, results on SNPs associated with cannabis and cocaine will be discussed together.

Variants in the endocannabinoid system

The endocannabinoid system is a signaling system widely expressed throughout the body. It is very complex and takes part in many cognitive and physiological processes, such as the regulation and modulation of learning and memory, food intake, nociception, motor coordination, reward processes and emotional control (Ameri 1999; López-Moreno, Echeverry-Alzate & Bühler 2012). The implication of the endocannabinoid system in these processes reveals its strong relationship to the dopamine system and therefore makes it a good candidate for studies on the genetics of drug abuse (for a review of the endocannabinoid system, its compounds and function, see Battista *et al.* 2012; Mechoulam & Parker 2013). Several studies have linked variants of the *CNR1* gene to drug addiction phenotypes, mainly cannabis consumption and dependence. Here, the SNP rs806380 is one of the most associated polymorphisms with problematic cannabis use in a sample of young people of different ethnicities (Hopfer *et al.* 2006) and cannabis dependence in European-Americans (Agrawal *et al.* 2009). To date, the molecular mechanism by which this SNP influences cannabis addiction has not been described, but these studies indicate that the minor allele G has a protective effect on cannabis dependence and problematic cannabis use.

Another SNP of the *CNR1* gene has been repeatedly associated with cocaine dependence. The rs806368 SNP was significantly associated with the cocaine addiction phenotype in European-Americans and African-Americans, although the association in African-Americans is controversial (Zuo *et al.* 2009; Clarke *et al.* 2013): Zuo *et al.* (2009) found a clear association between cocaine dependence and the rs806368 SNP in European-American family and case–control samples, but for African-Americans, the association was only significant in the family sample. This was replicated by Clarke *et al.* (2013), who found an initially significant association between rs806368 and cocaine dependence in an African-American case–control sample, but when combining their data with those from Zuo *et al.* (2009) to perform a meta-analysis, rs806368 was no longer significant in African-Americans (Clarke *et al.* 2013). As for rs806380, no specific biological mechanism has been found that clarifies how rs806368 influences drug addiction. Clarke *et al.* (2013) gave a tentative explanation, suggesting that because the SNP lies in an evolutionary conserved region, it may affect the stability of the *CNR1* mRNA transcript, reducing in this way its rate of translation and producing biological consequences relevant for cocaine addiction (Clarke *et al.* 2013).

Aside from the cannabinoid receptors and various endocannabinoid ligands, the endocannabinoid system is composed of two main enzymes (Piomelli 2003, 2004; Pertwee 2006). One of these enzymes is the fatty acid amide hydrolase (FAAH), which is encoded by the homonym gene *FAAH*. The FAAH enzyme is responsible mainly for degradation of the endogenous cannabinoid anandamide. It is widely expressed in the brain as well as in some peripheral tissues such as the pancreas and kidneys (Giang & Cravatt 1997). In relation to drug addiction, studies mainly focus on one particular SNP, rs324420. This is the only common missense variant in the *FAAH* gene with a MAF > 5 percent (Camilleri *et al.* 2008) and causes a change from the amino acid proline (Pro) to threonine (Thr). Studies indicate that this change in configuration of the amino acid chain has an important impact on the biochemical and cellular functioning of the enzyme. Sipe *et al.* (2002) showed that the presence of the minor allele A in homozygosis led to increased enzyme susceptibility to proteolysis, although the functional properties remained unchanged. Two years later, Chiang *et al.* (2004) studied the expression and activity of the FAAH protein in human peripheral T lymphocytes and showed that in subjects with the AA genotype, both the expression and the activity of the FAAH enzyme were reduced by half in comparison to CC/CA genotype subjects (Chiang *et al.* 2004). The authors showed that this reduction in protein expression was due neither to the cell types used nor to a reduction in mRNA levels, indicating that the decreased *FAAH* expression was more likely to be due to a post-transcriptional or post-translational mechanism that affects folding of the protein (Chiang *et al.* 2004). In addition to the mentioned biochemical differences, genetics techniques have shown that A allele carriers exhibited a lower threat-related and a higher reward-related amygdala reactivity compared with the carriers of the C allele (Hariri *et al.* 2009). In concordance with these data, Hariri *et al.* (2009) found a significant association between the A allele and lower anxiety and higher impulsivity compared with the C allele. This high impulsivity trait may confer A allele carriers greater risk for drug addiction. However, studies on which of the alleles (A or C) or genotypes (CC/CA/AA) increases or decreases vulnerability to drug addiction are contradictory. On the one hand, the A allele has been associated with risk for street drug abuse (Sipe *et al.* 2002) and antisocial personality disorders in alcoholics (Hoenicka *et al.* 2007), and the AA genotype has been related to regular use of sedatives in Caucasians (Tyndale *et al.* 2007). On the other hand, CC genotypes have been associated with more severe withdrawal symptoms and craving for marijuana (Haughey *et al.* 2008a; Schacht, Selling & Hutchison 2009) and marijuana addiction (Tyndale *et al.* 2007). In addition, Filbey *et al.* (2010) reported that C allele carriers, and not the A allele carriers, showed higher neural reactivity to drug-related cues (e.g. marihuana) in brain areas involved in reward and drug addiction. Until the specific biochemical background on how rs324420 influences drug addiction is discovered, it will be difficult to determine which of these alleles and genotypes confer a major risk for addiction.

Variants in the CHRNA5 *gene*

In addition to nicotine dependence, there is also evidence that links the non-synonymous SNP rs16969968 of the *CHRNA5* gene to cocaine addiction. This association was first reported by Saccone *et al.* (2008), who found an association between a small group of SNPs (including rs16969968) in the *CHRNA5-CHRNA3-CHRNB4* cluster and cocaine dependence in a sample of European-Americans and African-Americans. Later in that year, Grucza *et al.* (2008) demonstrated an association between this SNP and cocaine dependence in two independent samples of individuals of European descent. Interestingly, the minor allele A, which was the risk allele for nicotine addiction, appeared to be protective against cocaine dependence because it was present in a higher frequency in the non-cocaine dependence control group. These results were successfully replicated in an independent study carried out by Sherva *et al.* (2010). Because these results are very promising, more studies regarding rs16969968 and cocaine addiction must be conducted to

further confirm this association and explore possible common pathways for cocaine and nicotine addiction. In the following section, we will focus particularly on these common pathways of drug addiction.

Finally, very recently, a GWAS has found novel risk SNPs for cocaine dependence. One of these SNPs was the rs2629540 at the family with sequence similarity 53, member B gene (*FAM53B*) in Europeans and in African-Americans (Gelernter *et al.* 2014b). According to the authors, this gene maps to a chromosomal region which has previously been associated to cocaine dependence in previous studies. This SNP is therefore a valuable new candidate risk loci for cocaine dependence.

Common SNPs underlying drug addiction: nicotinic receptors and the endocannabinoid system

As seen throughout this review, some SNPs and genes are associated with more than one drug addiction.

One of these shared genes is *CHRNA5*. SNPs in this gene were significantly associated with nicotine-, cocaine- and alcohol-related phenotypes [see Tables 2–4 and the sections Variants in the serotonin receptor 1B (*HTR1B*) gene, Variants in *nAChR* genes and Variants in the *CHRNA5* gene]. Alcohol and nicotine act as nAChR agonists, enhancing opening of the channel and allowing entry of cations such as sodium and calcium. The α5 subunit has increased receptor desensitization and calcium permeability. Some studies suggest that the higher calcium entrance may activate several signal transduction pathways that cause gene activation or cell proliferation, biological processes highly involved in substance addiction and cancer, respectively (Wang *et al.* 2009). In fact, pre-clinical studies in mice have shown that the α5 subunit has an important role in nAChR subtypes that regulate dopamine transmission in the dorsal striatum (Exley *et al.* 2012). Therefore, perhaps the presence of polymorphisms in this subunit more strongly affects receptor response to agonists than polymorphisms in other nicotine subunits. Nevertheless, although both alcohol and nicotine dependence may be affected by variation in the *CHRNA5* gene, it remains unclear if the responsible polymorphisms are shared. Initial studies indicate that polymorphisms in the *CHRNA5* gene influencing risk for alcohol dependence are different from that influencing nicotine dependence. Wang *et al.* (2009) demonstrated an association between a group of SNPs in the *CHRNA5-CHRNA3-CHRNB4* gene cluster (including rs588765 and rs3841324) and alcohol dependence. Minor alleles of the same were shown to alter CHRNA5 expression in human post-mortem frontal cortex. Nevertheless, these SNPs were independent of those associated with nicotine dependence (such as rs16969968). On the contrary, Joslyn *et al.* (2008) showed that rs1051730 and rs8034191, which are strongly related to nicotine addiction and smoking behavior, were associated with the degree of alcohol response in humans. They concluded that the minor alleles of these SNPs (A and C, respectively) significantly reduced the level of response to alcohol and increased the risk of alcohol dependence.

The relationship between cocaine and the cholinergic system is less intuitive. Whereas nicotine and alcohol are direct agonists of nAChR, the effect of cocaine on these receptors in the brain appears to act primarily through dopamine projections. Administration of cocaine increases acetylcholine in the nucleus accumbens and in the dorsal striatum via VTA dopamine afferents upon striatal acetylcholine interneurons (for a detailed review of the relationship between cocaine addiction and acetylcholine, see Williams & Adinoff 2008). The effect of the rs16969968 polymorphism on cocaine addiction is not fully understood, and more molecular studies are needed. Nevertheless, Grucza *et al.* (2008) provided an elegant hypothetical explanation for the opposite effect of the minor allele of this SNP on nicotine and cocaine addiction, relying on the location of nAChR in the mesolimbic dopamine system. α5 nAChR subunits can be located on excitatory dopaminergic neurons as well as on inhibitory GABAergic neurons. As the minor allele A reduces receptor function, for nicotine, it leads to reduced nicotine-stimulated GABA transmission and enhanced dopamine response to nicotine, increasing the risk for nicotine addiction. For cocaine, the reduction of nAChR in dopaminergic neurons may reduce dopamine functioning and reduce vulnerability to cocaine addiction (Grucza *et al.* 2008). Changing the scope from biological pathways to underlying personality, Zayats *et al.* (2013) explored the interaction between personality domains and the rs16969968 SNP on the risk for nicotine dependence, cocaine dependence and cocaine-induced paranoia. They showed that cocaine-dependent participants with at least one protective allele against cocaine dependence (A allele) scored significantly higher on a personality domain called *conscientiousness* compared with homozygotic carriers of the risk allele. This personality domain has frequently been related to different drug dependencies, and high scores in *conscientiousness* are related to higher self-control, determination and organization. Nevertheless, no significant results were observed in nicotine-dependent participants.

These findings clearly suggest that variation in nicotinic cholinergic receptors is the common underlying biological risk factor for drug addiction, particularly polymorphisms in the *CHRNA5* gene, but it seems that specific variants in this gene may act differently for different forms of addiction.

Another common biological pathway for different dependencies is the endocannabinoid system. Its gene

variants have been related to alcohol, cannabis and also to cocaine addiction phenotypes. While in this study, no variants in the endocannabinoid system were highly significantly associated with alcohol and nicotine, it is established that the endocannabinoid system is implicated in these phenotypes (for a review on the implication of the endocannabinoid system and human drug addiction, see López-Moreno *et al.* 2012). Whereas cannabis is a direct agonist of CB1 receptors, alcohol and nicotine increase endocannabinoids most likely by increasing dopamine firing rate acting on GABAergic, glutamatergic and cholinergic interneurons, inducing anandamide release in the VTA (for a review, see Maldonado, Valverde & Berrendero 2006; Pava & Woodward 2012). To date, the neuropharmacological mechanism by which cocaine affects the endocannabinoid system is not clearly understood (for a review on the relationship between endocannabinoid transmission and cocaine addiction, see Arnold 2005), but several pre-clinical studies indicate a crucial role for the endocannabinoid system in neuroplasticity associated with development of cocaine addiction (Orio *et al.* 2009) and behavioral and neurochemical cocaine sensitization (Mereu *et al.* 2013).

As mentioned earlier in the section Variants in the endocannabinoid system, there is a clear lack of research regarding implication of variants in the endocannabinoid system on drug addiction, so that we can only speculate on how these variants may affect drug addiction, and if there are shared variants. The highlighted SNPs, rs806380 and rs324420 for cannabis, and rs806368 for cocaine dependence, have been shown not to be in LD with each other, so we may estimate that this SNP acts independently on this phenotypes. Nevertheless, as mentioned in the section Variants in the endocannabinoid system, the rs324420 SNP in the *FAAH* gene has been linked to several different types of addiction such as alcohol (Sipe *et al.* 2002; Hoenicka *et al.* 2007; Bühler *et al.* 2014), sedatives and marijuana (Tyndale *et al.* 2007; Haughey *et al.* 2008b; Schacht *et al.* 2009,) cocaine and polysubstance abuse (Flanagan *et al.* 2006), but few of these associations were reliably replicated. Moreover, there is an important controversy over which of the SNP alleles confers addiction risk because in different studies of the same drug, risk has been linked to different alleles (Sipe *et al.* 2002; Bühler *et al.* 2014). Therefore, it appears that the influence of rs324420 is quite complex and requires more specific biochemical studies.

Without a doubt, as well as the cholinergic system, the endocannabinoid system is the most common pathway for different drug dependencies, but clearly more research is needed to address the influence of individual genetic variants on addiction.

Finding common pathways for addiction is a very complex task and a goal of future addiction genetic research. Several studies and reviews have been published to this end (i.e. Nestler 2002, 2005; Le Moal & Koob 2007). Li, Mao & Wei (2008) published a very interesting study in which five main pathways of different drug dependencies were identified and integrated into a hypothetical common molecular network for addiction. It will be interesting with the rise of new sequencing and imaging technologies if research in the near future will clearly identify these common pathways and flesh out the role of genetic variation in these circuits.

DISCUSSION

What can SNPs really tell us about drug addiction?

Here, we have reviewed common single nucleotide gene variants that were most significantly associated with alcohol-, nicotine-, cannabis- and cocaine-related phenotypes. To select these SNPs, we extracted significant association results of 371 studies published between the years of 2000 and 2012 and filtered the genes and tagging SNPs that were replicated at least once. We then applied the Fisher method for combining *P*-values and selected the 20 most significant SNPs associated with alcohol- and nicotine-related phenotypes and the two most significant SNPs associated with cannabis and cocaine phenotypes. Interestingly, very few of the top 20 or even the top 2 SNPs have more than two or three replication studies. Difficulties in replicating results have always been a major issue in genetic association studies (Colhoun, McKeigue & Davey Smith 2003; Vrieze, Iacono & McGue 2012). There are many reasons why replication can fail, including variability in phenotype definition, inappropriate selection of study subjects, sample size, data quality or a lack of appropriate statistical analysis (Colhoun *et al.* 2003; Greene *et al.* 2009). All these issues can affect the statistical power of an association study. In fact, inappropriate statistical power as well as insufficient stringent significance thresholds are common problems in human genetic studies and are believed to be a frequent reason for the low replication success (for a review of statistical power in genetic studies, see Sham and Purcell 2014). On the contrary, the demanding *P*-values required in GWAS may omit positive associations with disease susceptibility gene variants that present only moderate *P*-values (Li *et al.* 2012). Finally, the possibility of false-negative/false-positive results in the initial study should also be considered when difficulties in replication arise. Again, lack of statistical power can be the cause of false-negative as well as false-positive results.

However, replication failure may also be a sign of the integral genetic heterogeneity underlying common complex diseases such as drug addiction. Some authors have noted that different individuals might develop the

same addiction disease (i.e. alcohol addiction) via different pathways that start from different combinations of disease susceptibility alleles (Palmer *et al.* 2012). This has been frequently observed when comparing different ethnic groups or samples from different geographic locations (Li & Burmeister 2009). Several gene variants that were significantly related to an addiction phenotype in ethnic population group were not related in other groups. Studies have shown that different groups present different minor allele frequencies of SNPs, and therefore, the underlying genetic pathways related to addiction are ethnically and geographically dependent. However, some authors have suggested that genetic heterogeneity exists on smaller scales than ethnic groups. McClellan & King (2010) indicated that 'complex human diseases are large collections of individually rare, even private, conditions', remarking the importance of genetic heterogeneity at the individual level. As stated by these authors, an illness in every affected individual may arise from different specific causes, so that replication of gene studies may only emerge after identifying biologically relevant variations and mutations that disrupt addiction-related biological processes. Therefore, these authors noted the importance of rare and biologically significant variants in common complex disease, issues that cannot be addressed by GWAS or the majority of candidate gene studies.

A main limitation of GWAS and CGAS is the lack of insight that is provided into the biologic pathway that could provide a causative link to the disease (Kendler 2013). As seen in this review, association studies have identified hundreds of SNPs that are statistically correlated with different addiction phenotypes, but very few have an identified biological relevance that could underlie drug addiction. Biological relevance appears to be a key issue in the replicated SNPs. Many of the most significant and replicated SNPs included in this review are functional non-synonymous SNPs – SNPs located in coding regions causing an amino acid variation in the protein product. As a consequence, these SNPs are most likely to have an impact on phenotype (Sunyaev *et al.* 2001; Ramensky *et al.* 2002). Sunyaev *et al.* (2001) estimated that approximately 20 percent of common human non-synonymous SNPs damage the protein, so that the average human genotype carries approximately 2000 deleterious SNPs. All these SNPs together may have a deleterious impact on fitness. It is important to note that although most replication of these non-synonymous SNPs came from candidate gene studies, several recent GWAS confirm these associations (e.g. see Park *et al.* 2013 for the rs12229984 and alcohol dependence, or Lips *et al.* 2010 for the rs16969968 and nicotine dependence).

Nevertheless, the majority of SNPs identified by GWAS, including addiction GWAS, lie in non-coding regions such as introns and promoters. As seen in Tables 2 and 3, of the 20 SNPs most significantly associated with the alcohol- and nicotine-related phenotypes described in this study, most belong to intronic or 5′3′-flanking regions. These non-coding regions have important roles in regulating alternative splicing and gene expression, so genetic variants in these regions may affect these functional properties. As previously stated, to date, non-coding variants have been seriously underestimated, but because they frequently arise in GWAS, there are many potentially functional non-coding SNPs. In fact, functional non-coding SNPs have been identified and associated with several disease phenotypes (for a review, see Knight 2003) including alcohol and nicotine addiction (Flora *et al.* 2013; Gallego *et al.* 2013) where these variants mainly influence gene transcription and expression. However, the possibility remains that most of these non-coding variants are related to these phenotypes because of LD with a functional SNP (for a review on functional intronic gene variants, see Cooper 2010).

Despite the fact that some variants have been successfully replicated, the majority of identified common variants explain only a small fraction of estimated heritability. Although a GWAS with a sample size of 2000–5000 is able to identify common variants with an odds ratio of at least 1.5 (Altshuler, Daly & Lander 2008), to date, no complex disease, including addiction, has more than 10 percent of its genetic variance explained by common variants. Massive sample sizes of GWAS are required to explain 20 percent or more of genetic variation due to common variants that underlie these diseases. It has been estimated that 60 000–100 000 individuals will be needed to capture almost 15 percent of variance underlying any given phenotype (Frazer *et al.* 2009). This means that using common variance, most of the heritable fraction estimated by twin and family studies for addiction is missing. Several authors have suggested that part of this missing heritability may be found in rare variants, claiming a shift from the common variants-common-disease model to the rare-variants common-disease model (Frazer *et al.* 2009; McClellan & King 2010; Wang *et al.* 2014). The assumption is that common diseases are the result of a summation of effects of low-frequency dominantly and independently acting high-penetrance variants (rare variant hypothesis) rather than the consequence of a combination of low-penetrant high-frequency variants (common variant hypothesis). The implication of rare variants is sensible in the light of evolution theory. Mutations with deleterious effects would be prevented from transmission to subsequent generation due to natural selection, being mostly of recent origin and individually very rare (McClellan & King 2010; Wang *et al.* 2014). Although this may apply

for a series of early onset diseases that have an important impact on reproduction and fertility, it may not be the case for later-onset diseases such as drug addiction. Nevertheless, the development of new sequencing methods over the last few years has allowed focus on rare variants in addiction research and have revealed significant associations between rare variants and nicotine (Wessel *et al.* 2010; Xie *et al.* 2011), alcohol (Zuo *et al.* 2013) and opioid dependence (Xie *et al.* 2014; for a review on new sequencing methods and rare variant association in addiction research, see Wang *et al.* 2014). Rare variants should therefore be taken into account for future work on the missing heritability and heterogeneity of addiction. However, common variance should not be discarded entirely.

The results of GWAS and candidate studies have identified an important part of the genetic background that explains heritability of addiction. These techniques allowed for the determination of the first gene variants that may be partially responsible for the development of an addiction disease. Nevertheless, as stated by multiple authors, the most frequently replicated SNPs at present are most likely those with the major implication and larger effect size in addiction variability (Frazer *et al.* 2009; Vrieze *et al.* 2014). Future work should emphasize disentangling the biological relevance and molecular functioning of gene variants so that potentially causative pathways for drug addiction can be uncovered. The implication of epistatic factors, epigenetic changes and gene–environment interactions make this task even more challenging. Translational and reverse translational research methods will be useful tools for a better understanding of the impact of gene variants on biological networks involved in addiction.

Acknowledgements

This work was supported by the Fondo de Investigación Sanitaria (Red de Trastornos Adictivos, FEDER, RD12/0028/0015 to J.A.L.M and RD12/0028/0001 to F.R. de F), Ministerio de Ciencia e Innovación (SAF2011-26818 to J.A.L.M.) and The European Foundation for Alcohol Research (EA 12 21 to J.A.L.M.).

Disclosure/Conflict of Interest

None.

Authors Contribution

JALM and KMB were responsible for the study concept and design. KMB, EG, VEA and JCC contributed to the bibliographical research. KMB and JALM wrote the manuscript. FRdF was responsible for critical review of the manuscript and valuable comments. All authors reviewed the content and approved the final version of this manuscript. All authors critically reviewed content and approved final version for publication.

References

Agrawal A, Lynskey MT, Hinrichs A, Grucza R, Saccone SF, Krueger R, Neuman R, Howells W, Fisher S, Fox L, Cloninger R, Dick DM, Doheny KF, Edenberg HJ, Goate AM, Hesselbrock V, Johnson E, Kramer J, Kuperman S, Nurnberger JI Jr., Pugh E, Schuckit M, Tischfield J, GENEVA Consortium, Rice JP, Bucholz KK, Bierut LJ (2011) A genome-wide association study of DSM-IV cannabis dependence. Addict Biol 16:514–518.

Agrawal A, Pergadia ML, Saccone SF, Hinrichs AL, Lessov-Schlaggar CN, Saccone NL, Neuman RJ, Breslau N, Johnson E, Hatsukami D, Montgomery GW, Heath AC, Martin NG, Goate AM, Rice JP, Bierut LJ, Madden PA (2008) Gamma-aminobutyric acid receptor genes and nicotine dependence: evidence for association from a case-control study. Addiction 103:1027–1038.

Agrawal A, Wetherill L, Dick DM, Xuei X, Hinrichs A, Hesselbrock V, Kramer J, Nurnberger JI Jr., Schuckit M, Bierut LJ, Edenberg HJ, Foroud T (2009) Evidence for association between polymorphisms in the cannabinoid receptor 1 (CNR1) gene and cannabis dependence. Am J Med Genet B Neuropsychiatr Genet 150B:736–740.

Albert PR (2011) What is a functional genetic polymorphism? Defining classes of functionality. J Psychiatry Neurosci 36:363–365.

Albuquerque EX, Pereira EF, Alkondon M, Rogers SW (2009) Mammalian nicotinic acetylcholine receptors: from structure to function. Physiol Rev 89:73–120.

Altshuler D, Daly MJ, Lander ES (2008) Genetic mapping in human disease. Science 322:881–888.

Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, Peltonen L, Dermitzakis E, Bonnen PE, Altshuler DM, Gibbs RA, de Bakker PI, Deloukas P, Gabriel SB, Gwilliam R, Hunt S, Inouye M, Jia X, Palotie A, Parkin M, Whittaker P, Yu F, Chang K, Hawes A, Lewis LR, Ren Y, Wheeler D, Gibbs RA, Muzny DM, Barnes C, Darvishi K, Hurles M, Korn JM, Kristiansson K, Lee C, McCarrol SA, Nemesh J, Dermitzakis E, Keinan A, Montgomery SB, Pollack S, Price AL, Soranzo N, Bonnen PE, Gibbs RA, Gonzaga-Jauregui C, Keinan A, Price AL, Yu F, Anttila V, Brodeur W, Daly MJ, Leslie S, McVean G, Moutsianas L, Nguyen H, Schaffner SF, Zhang Q, Ghori MJ, McGinnis R, McLaren W, Pollack S, Price AL, Schaffner SF, Takeuchi F, Grossman SR, Shlyakhter I, Hostetter EB, Sabeti PC, Adebamowo CA, Foster MW, Gordon DR, Licinio J, Manca MC, Marshall PA, Matsuda I, Ngare D, Wang VO, Reddy D, Rotimi CN, Royal CD, Sharp RR, Zeng C, Brooks LD, McEwen JE (2010) Integrating common and rare genetic variation in diverse human populations. Nature 467:52–58.

Ameri A (1999) The effects of cannabinoids on the brain. Prog Neurobiol 58:315–348.

Arnold JC (2005) The role of endocannabinoid transmission in cocaine addiction. Pharmacol Biochem Behav 81:396–406.

Baker TB, Weiss RB, Bolt D, von Niederhausern A, Fiore MC, Dunn DM, Piper ME, Matsunami N, Smith SS, Coon H, McMahon WM, Scholand MB, Singh N, Hoidal JR, Kim SY, Leppert MF, Cannon DS (2009) Human neuronal acetylcholine receptor A5-A3-B4 haplotypes are associated with multiple nicotine dependence phenotypes. Nicotine Tob Res 11:785–796.

Ball D (2008) Addiction science and its genetics. Addiction 103:360–367.

Battista N, Di Tommaso M, Bari M, Maccarrone M (2012) The endocannabinoid system: an overview. Front Behav Neurosci 6:9.

Bierut LJ (2010) Convergence of genetic findings for nicotine dependence and smoking related diseases with chromosome 15q24-25. Trends Pharmacol Sci 31:46–51.

Bierut LJ, Agrawal A, Bucholz KK, Doheny KF, Laurie C, Pugh E, Fisher S, Fox L, Howells W, Bertelsen S, Hinrichs AL, Almasy L, Breslau N, Culverhouse RC, Dick DM, Edenberg HJ, Foroud T, Grucza RA, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Krueger RF, Kuperman S, Lynskey M, Mann K, Neuman RJ, Nöthen MM, Nurnberger JI Jr, Porjesz B, Ridinger M, Saccone NL, Saccone SF, Schuckit MA, Tischfield JA, Wang JC, Rietschel M, Goate AM, Rice JP; Gene, Environment Association Studies Consortium (2010) A genome-wide association study of alcohol dependence. Proc Natl Acad Sci USA 107:5082–5087.

Bierut LJ, Goate AM, Breslau N, Johnson EO, Bertelsen S, Fox L, Agrawal A, Bucholz KK, Grucza R, Hesselbrock V, Kramer J, Kuperman S, Nurnberger J, Porjesz B, Saccone NL, Schuckit M, Tischfield J, Wang JC, Foroud T, Rice JP, Edenberg HJ (2012) ADH1B is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. Mol Psychiatry 17:445–450.

Bierut LJ, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau OF, Swan GE, Rutter J, Bertelsen S, Fox L, Fugman D, Goate AM, Hinrichs AL, Konvicka K, Martin NG, Montgomery GW, Saccone NL, Saccone SF, Wang JC, Chase GA, Rice JP, Ballinger DG (2007) Novel genes identified in a high-density genome wide association study for nicotine dependence. Hum Mol Genet 16:24–35.

Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Grucza RA, Xuei X, Saccone NL, Saccone SF, Bertelsen S, Fox L, Horton WJ, Breslau N, Budde J, Cloninger CR, Dick DM, Foroud T, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Kuperman S, Madden PA, Mayo K, Nurnberger J Jr., Pomerleau O, Porjesz B, Reyes O, Schuckit M, Swan G, Tischfield JA, Edenberg HJ, Rice JP, Goate AM (2008) Variants in nicotinic receptors and risk for nicotine dependence. Am J Psychiatry 165:1163–1171.

Blum K, Noble EP (1994) The sobering D2 story. Science 265:1346–1347.

Blum K, Noble EP, Sheridan PJ, Montgomery A, Ritchie T, Jagadeeswaran P, Nogami H, Briggs AH, Cohn JB (1990) Allelic association of human dopamine D2 receptor gene in alcoholism. JAMA 263:2055–2060.

Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L (1998) Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. Proc Natl Acad Sci USA 95:9608–9613.

Borràs E, Coutelle C, Rosell A, Fernández-Muixi F, Broch M, Crosas B, Hjelmqvist L, Lorenzo A, Gutiérrez C, Santos M, Szczepanek M, Heilig M, Quattrocchi P, Farrés J, Vidal F, Richart C, Mach T, Bogdal J, Jörnvall H, Seitz HK, Couzigou P, Parés X (2000) Genetic polymorphism of alcohol dehydrogenase in Europeans: the ADH2*2 allele decreases the risk for alcoholism and is associated with ADH3*1. Hepatology 31:984–989.

Bouwknecht JA, Hijzen TH, van der Gugten J, Maes RA, Hen R, Olivier B (2001) Absence of 5-HT(1B) receptors is associated with impaired impulse control in male 5-HT(1B) knockout mice. Biol Psychiatry 49:557–568.

Bühler KM, Huertas E, Echeverry-Alzate V, Giné E, Moltó E, Montoliu L, López-Moreno JA (2014) Risky alcohol consumption in young people is associated with the fatty acid amide hydrolase gene polymorphism C385A and affective rating of drug pictures. Mol Genet Genomics 289:279–289.

Camilleri M, Carlson P, McKinzie S, Grudell A, Busciglio I, Burton D, Baxter K, Ryks M, Zinsmeister AR (2008) Genetic variation in endocannabinoid metabolism, gastrointestinal motility, and sensation. Am J Physiol Gastrointest Liver Physiol 294:G13–G19.

Cao JX, Hu J, Ye XM, Xia Y, Haile CA, Kosten TR, Zhang XY (2011) Association between the 5-HTR1B gene polymorphisms and alcohol dependence in a Han Chinese population. Brain Res 1376:1–9.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807–821.

Chen X, Cho K, Singer BH, Zhang H (2011) The nuclear transcription factor PKNOX2 is a candidate gene for substance dependence in European-origin women. PLoS ONE 6:e16002.

Chiang KP, Gerber AL, Sipe JC, Cravatt BF (2004) Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. Hum Mol Genet 13:2113–2119.

Choi IG, Son HG, Yang BH, Kim SH, Lee JS, Chai YG, Son BK, Kee BS, Park BL, Kim LH, Choi YH, Shin HD (2005) Scanning of genetic effects of alcohol metabolism gene (ADH1B and ADH1C) polymorphisms on the risk of alcoholism. Hum Mutat 26:224–234.

Clarke TK, Bloch PJ, Ambrose-Lanci LM, Ferraro TN, Berrettini WH, Kampman KM, Dackis CA, Pettinati HM, O'Brien CP, Oslin DW, Lohoff FW (2013) Further evidence for association of polymorphisms in the CNR1 gene with cocaine addiction: confirmation in an independent sample and meta-analysis. Addict Biol 18:702–708.

Colhoun HM, McKeigue PM, Davey Smith G (2003) Problems of reporting genetic associations with complex outcomes. Lancet 361:865–872.

Cooper DN (2010) Functional intronic polymorphisms: buried treasure awaiting discovery within our genes. Hum Genomics 4:284–288.

Covault J, Gelernter J, Hesselbrock V, Nellissery M, Kranzler HR (2004) Allelic and haplotypic association of GABRA2 with alcohol dependence. Am J Med Genet B Neuropsychiatr Genet 129B:104–109.

Crabbe JC, Phillips TJ, Feller DJ, Hen R, Wenger CD, Lessov CN, Schafer GL (1996) Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors. Nat Genet 14:98–101.

di Giovine FS, Takhsh E, Blakemore AI, Duff GW (1992) Single base polymorphism at -511 in the human interleukin-1 beta gene (IL1 beta). Hum Mol Genet 1:450.

Dani JA, Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 47:699–729.

Dennis NA, Need AC, LaBar KS, Waters-Metenier S, Cirulli ET, Kragel J, Goldstein DB, Cabeza R (2010) COMT val108/158

met genotype affects neural but not cognitive processing in healthy individuals. Cereb Cortex 20:672–683.

Dickinson D, Elvevåg B (2009) Genes, cognition and brain through a COMT lens. Neuroscience 164:72–87.

Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV (2003) Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. Hum Mol Genet 12:205–216.

Duaux E, Krebs MO, Loo H, Poirier MF (2000) Genetic vulnerability to drug abuse. Eur Psychiatry 15:109–114.

Ducci F, Goldman D (2008) Genetic approaches to addiction: genes and alcohol. Addiction 103:1414–1428.

Edenberg HJ (2007) The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health 30:5–13.

Edenberg HJ (2012) Genes contributing to the development of alcoholism: an overview. Alcohol Res 34:336–338.

Edenberg HJ, Dick DM, Xuei X, Tian H, Almasy L, Bauer LO, Crowe RR, Goate A, Hesselbrock V, Jones K, Kwon J, Li TK, Nurnberger JI Jr, O'Connor SJ, Reich T, Rice J, Schuckit MA, Porjesz B, Foroud T, Begleiter H (2004) Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. Am J Hum Genet 74:705–714.

Edenberg HJ, Jerome RE, Li M (1999) Polymorphism of the human alcohol dehydrogenase 4 (ADH4) promoter affects gene expression. Pharmacogenetics 9:25–30.

Edenberg HJ, Xuei X, Chen HJ, Tian H, Wetherill LF, Dick DM, Almasy L, Bierut L, Bucholz KK, Goate A, Hesselbrock V, Kuperman S, Nurnberger J, Porjesz B, Rice J, Schuckit M, Tischfield J, Begleiter H, Foroud T (2006) Association of alcohol dehydrogenase genes with alcohol dependence: a comprehensive analysis. Hum Mol Genet 15:1539–1549.

Enoch MA, Waheed JF, Harris CR, Albaugh B, Goldman D (2006) Sex differences in the influence of COMT Val158Met on alcoholism and smoking in plains American Indians. Alcohol Clin Exp Res 30:399–406.

Eriksson M, Berggren U, Blennow K, Fahlke C, Månsson JE, Balldin J (2000) Alcoholics with the dopamine receptor DRD2 A1 allele have lower platelet monoamine oxidase-B activity than those with the A2 allele: a preliminary study. Alcohol Alcohol 35:493–498.

Exley R, McIntosh JM, Marks MJ, Maskos U, Cragg SJ (2012) Striatal α5 nicotinic receptor subunit regulates dopamine transmission in dorsal striatum. J Neurosci 32:2352–2356.

Filbey FM, Schacht JP, Myers US, Chavez RS, Hutchison KE (2010) Individual and additive effects of the CNR1 and FAAH genes on brain response to marijuana cues. Neuropsychopharmacology 35:967–975.

Fisher RA, Immer FR, Tedin O (1932) The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. Genetics 17:107–124.

Flanagan JM, Gerber AL, Cadet JL, Beutler E, Sipe JC (2006) The fatty acid amide hydrolase 385 A/A (P129T) variant: haplotype analysis of an ancient missense mutation and validation of risk for drug addiction. Hum Genet 120:581–588.

Flora AV, Zambrano CA, Gallego X, Miyamoto JH, Johnson KA, Cowan KA, Stitzel JA, Ehringer MA (2013) Functional characterization of SNPs in CHRNA3/B4 intergenic region associated with drug behaviors. Brain Res 1529:1–15.

Fowler CD, Lu Q, Johnson PM, Marks MJ, Kenny PJ (2011) Habenular α5 nicotinic receptor subunit signaling controls nicotine intake. Nature 471:597–601.

Frazer KA, Murray SS, Schork NJ, Topol EJ (2009) Human genetic variation and its contribution to complex traits. Nat Rev Genet 10:241–251.

Furay AR, Neumaier JF, Mullenix AT, Kaiyala KK, Sandygren NK, Hoplight BJ (2011) Overexpression of 5-HT(1B) mRNA in nucleus accumbens shell projection neurons differentially affects microarchitecture of initiation and maintenance of ethanol consumption. Alcohol 45:19–32.

Gabby M, Tauber M, Porat S, Simantov R (1996) Selective role of glutathione in protecting human neuronal cells from dopamine-induced apoptosis. Neuropharmacology 35:571–578.

Gallego X, Cox RJ, Laughlin JR, Stitzel JA, Ehringer MA (2013) Alternative CHRNB4 3′-UTRs mediate the allelic effects of SNP rs1948 on gene expression. PLoS ONE 8:e63699.

Gelernter J, Kranzler HR, Sherva R, Almasy L, Koesterer R, Smith AH, Anton R, Preuss UW, Ridinger M, Rujescu D, Wodarz N, Zill P, Zhao H, Farrer LA (2014a) Genome-wide association study of alcohol dependence: significant findings in African- and European-Americans including novel risk loci. Mol Psychiatry 19:41–49.

Gelernter J, Sherva R, Koesterer R, Almasy L, Zhao H, Kranzler HR, Farrer L (2014b) Genome-wide association study of cocaine dependence and related traits: FAM53B identified as a risk gene. Mol Psychiatry 19:717–723.

Giang DK, Cravatt BF (1997) Molecular characterization of human and mouse fatty acid amide hydrolases. Proc Natl Acad Sci USA 94:2238–2242.

Gizer IR, Edenberg HJ, Gilder DA, Wilhelmsen KC, Ehlers CL (2011) Association of alcohol dehydrogenase genes with alcohol-related phenotypes in a Native American community sample. Alcohol Clin Exp Res 35:2008–2018.

Goedde HW, Agarwal DP, Fritze G, Meier-Tackmann D, Singh S, Beckmann G, Bhatia K, Chen LZ, Fang B, Lisker R, Paik YK, Rothhammer F, Saha N, Segal B, Srivastava LM, Czeizel A (1992) Distribution of ADH2 and ALDH2 genotypes in different populations. Hum Genet 88:344–346.

Gorwood P, Le Strat Y, Ramoz N, Dubertret C, Moalic JM, Simonneau M (2012) Genetics of dopamine receptors and drug addiction. Hum Genet 131:803–822.

Greene CS, Penrod NM, Williams SM, Moore JH (2009) Failure to replicate a genetic association may provide important clues about genetic architecture. PLoS ONE 4:e5639.

Grucza RA, Wang JC, Stitzel JA, Hinrichs AL, Saccone SF, Saccone NL, Bucholz KK, Cloninger CR, Neuman RJ, Budde JP, Fox L, Bertelsen S, Kramer J, Hesselbrock V, Tischfield J, Nurnberger JI Jr., Almasy L, Porjesz B, Kuperman S, Schuckit MA, Edenberg HJ, Rice JP, Goate AM, Bierut LJ (2008) A risk allele for nicotine dependence in CHRNA5 is a protective allele for cocaine dependence. Biol Psychiatry 64:922–929.

Guindalini C, Scivoletto S, Ferreira RG, Breen G, Zilberman M, Peluso MA, Zatz M (2005) Association of genetic variants in alcohol dehydrogenase 4 with alcohol dependence in Brazilian patients. Am J Psychiatry 162:1005–1007.

Hall SK, Perregaux DG, Gabel CA, Woodworth T, Durham LK, Huizinga TW, Breedveld FC, Seymour AB (2004) Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein. Arthritis Rheum 50:1976–1983.

Han Y, Gu S, Oota H, Osier MV, Pakstis AJ, Speed WC, Kidd JR, Kidd KK (2007) Evidence of positive selection on a class I ADH locus. Am J Hum Genet 80:441–456.

Hariri AR, Gorka A, Hyde LW, Kimak M, Halder I, Ducci F, Ferrell RE, Goldman D, Manuck SB (2009) Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. Biol Psychiatry 66:9–16.

Haughey HM, Marshall E, Schacht JP, Louis A, Hutchison KE (2008a) Marijuana withdrawal and craving: influence of the cannabinoid receptor 1 (CNR1) and fatty acid amide hydrolase (FAAH) genes. Addiction 103:1678–1686.

Haughey HM, Ray LA, Finan P, Villanueva R, Niculescu M, Hutchison KE (2008b) Human gamma-aminobutyric acid A receptor alpha2 gene moderates the acute effects of alcohol and brain mRNA expression. Genes Brain Behav 7:447–454.

Heilig M, Goldman D, Berrettini W, O'Brien CP (2011) Pharmacogenetic approaches to the treatment of alcohol addiction. Nat Rev Neurosci 12:670–684.

Hill SY, Hoffman EK, Zezza N, Thalamuthu A, Weeks DE, Matthews AG, Mukhopadhyay I (2008) Dopaminergic mutations: within-family association and linkage in multiplex alcohol dependence families. Am J Med Genet B Neuropsychiatr Genet 147B:517–526.

Hill SY, Locke J, Zezza N, Kaplan B, Neiswanger K, Steinhauer SR, Wipprecht G, Xu J (1998) Genetic association between reduced P300 amplitude and the DRD2 dopamine receptor A1 allele in children at high risk for alcoholism. Biol Psychiatry 43:40–51.

Hillemacher T, Heberlein A, Muschler MA, Bleich S, Frieling H (2011) Opioid modulators for alcohol dependence. Expert Opin Investig Drugs 20:1073–1086.

Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K (2002) A comprehensive review of genetic association studies. Genet Med 4:45–61.

Hirvonen M, Laakso A, Någren K, Rinne JO, Pohjalainen T, Hietala J (2004) C957T polymorphism of the dopamine D2 receptor (DRD2) gene affects striatal DRD2 availability *in vivo*. Mol Psychiatry 9:1060–1061. Erratum in: Mol Psychiatry 2005 Sep;10(9):889.

Hoenicka J, Ponce G, Jiménez-Arriero MA, Ampuero I, Rodríguez-Jiménez R, Rubio G, Aragüés M, Ramos JA, Palomo T (2007) Association in alcoholic patients between psychopathic traits and the additive effect of allelic forms of the CNR1 and FAAH endocannabinoid genes, and the 3′ region of the DRD2 gene. Neurotox Res 11:51–60.

Hoenicka J, Quiñones-Lombraña A, España-Serrano L, Alvira-Botero X, Kremer L, Pérez-González R, Rodríguez-Jiménez R, Jiménez-Arriero MA, Ponce G, Palomo T (2010) The ANKK1 gene associated with addictions is expressed in astroglial cells and upregulated by apomorphine. Biol Psychiatry 67:3–11.

Honea R, Verchinski BA, Pezawas L, Kolachana BS, Callicott JH, Mattay VS, Weinberger DR, Meyer-Lindenberg A (2009) Impact of interacting functional variants in COMT on regional gray matter volume in human brain. Neuroimage 45:44–51.

Hopfer CJ, Young SE, Purcell S, Crowley TJ, Stallings MC, Corley RP, Rhee SH, Smolen A, Krauter K, Hewitt JK, Ehringer MA (2006) Cannabis receptor haplotype associated with fewer cannabis dependence symptoms in adolescents. Am J Med Genet B Neuropsychiatr Genet 141B:895–901.

Hu J, Henry S, Gallezot JD, Ropchan J, Neumaier JF, Potenza MN, Sinha R, Krystal JH, Huang Y, Ding YS, Carson RE, Neumeister A (2010) Serotonin 1B receptor imaging in alcohol dependence. Biol Psychiatry 67:800–803.

Hurley TD, Edenberg HJ (2012) Genes encoding enzymes involved in ethanol metabolism. Alcohol Res 34:339–344.

Ittiwut C, Yang BZ, Kranzler HR, Anton RF, Hirunsatit R, Weiss RD, Covault J, Farrer LA, Gelernter J (2012) GABRG1 and GABRA2 variation associated with alcohol dependence in African Americans. Alcohol Clin Exp Res 36:588–593.

Jackson KJ, Marks MJ, Vann RE, Chen X, Gamage TF, Warner JA, Damaj MI (2010) Role of alpha5 nicotinic acetylcholine receptors in pharmacological and behavioral effects of nicotine in mice. J Pharmacol Exp Ther 334:137–146.

Johnson AD (2009) Single-nucleotide polymorphism bioinformatics: a comprehensive review of resources. Circ Cardiovasc Genet 2:530–536.

Johnson AD, Handsaker RE, Pulit S, Nizzari MM, O'Donnell CJ, de Bakker PIW (2008) SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics 24:938–2939.

Joslyn G, Brush G, Robertson M, Smith TL, Kalmijn J, Schuckit M, White RL (2008) Chromosome 15q25.1 genetic markers associated with level of response to alcohol in humans. Proc Natl Acad Sci USA 105:20368–20373.

Jönsson EG, Nöthen MM, Grünhage F, Farde L, Nakashima Y, Propping P, Sedvall GC (1999) Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. Mol Psychiatry 4:290–296.

Kendler KS (2013) What psychiatric genetics has taught us about the nature of psychiatric illness and what is left to learn. Mol Psychiatry 18:1058–1066.

Kimura M, Miyakawa T, Matsushita S, So M, Higuchi S (2011) Gender differences in the effects of ADH1B and ALDH2 polymorphisms on alcoholism. Alcohol Clin Exp Res 35:1923–1927.

Knight JC (2003) Functional implications of genetic variation in non-coding DNA for disease susceptibility and gene regulation. Clin Sci (Lond) 104:493–501.

Koob GF (2004) A role for GABA mechanisms in the motivational effects of alcohol. Biochem Pharmacol 68:1515–1525.

Kovanen L, Saarikoski ST, Haukka J, Pirkola S, Aromaa A, Lönnqvist J, Partonen T (2010) Circadian clock gene polymorphisms in alcohol use disorders and alcohol consumption. Alcohol Alcohol 45:303–311.

Kroslak T, Laforge KS, Gianotti RJ, Ho A, Nielsen DA, Kreek MJ (2007) The single nucleotide polymorphism A118G alters functional properties of the human mu opioid receptor. J Neurochem 103:77–87.

Laakso A, Pohjalainen T, Bergman J, Kajander J, Haaparanta M, Solin O, Syvälahti E, Hietala J (2005) The A1 allele of the human D2 dopamine receptor gene is associated with increased activity of striatal L-amino acid decarboxylase in healthy subjects. Pharmacogenet Genomics 15:387–391.

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6:243–250.

Laine TP, Ahonen A, Räsänen P, Pohjalainen T, Tiihonen J, Hietala J (2001) The A1 allele of the D2 dopamine receptor gene is associated with high dopamine transporter density in detoxified alcoholics. Alcohol Alcohol 36:262–265.

Lander ES, Schork NJ (1994) Genetic dissection of complex traits. Science 265:2037–2048.

Lappalainen J, Kranzler HR, Malison R, Price LH, Van Dyck C, Rosenheck RA, Cramer J, Southwick S, Charney D, Krystal J,

Gelernter J (2002) A functional neuropeptide Y Leu7Pro polymorphism associated with alcohol dependence in a large population sample from the United States. Arch Gen Psychiatry 59:825–831.

Latella MC, Di Castelnuovo A, de Lorgeril M, Arnout J, Cappuccio FP, Krogh V, Siani A, van Dongen M, Donati MB, de Gaetano G, Iacoviello L, European Collaborative, Li D, Zhao H, Gelernter J (2012) Further clarification of the contribution of the ADH1C gene to vulnerability of alcoholism and selected liver diseases. Hum Genet 131:1361–1374.

Le Moal M, Koob GF (2007) Drug addiction: pathways to the disease and pathophysiological perspectives. Eur Neuropsychopharmacol 17:377–393.

Lee SY, Lin WW, Huang SY, Kuo PH, Wang CL, Wu PL, Chen SL, Wu JY, Ko HC, Lu RB (2009) The relationship between serotonin receptor 1B polymorphisms A-161T and alcohol dependence. Alcohol Clin Exp Res 33:1589–1595.

Li CY, Mao X, Wei L (2008) Genes and (common) pathways underlying drug addiction. PLoS Comput Biol 4:e2.

Li D, Zhao H, Gelernter J (2011) Strong association of the alcohol dehydrogenase 1B gene (ADH1B) with alcohol dependence and alcohol-induced medical diseases. Biol Psychiatry 70:504–512.

Li MD, Burmeister M (2009) New insights into the genetics of addiction. Nat Rev Genet 10:225–231.

Li MJ, Wang P, Liu X, Lim EL, Wang Z, Yeager M, Wong MP, Sham PC, Chanock SJ, Wang J (2012) GWASdb: a database for human genetic variants identified by genome-wide association studies. Nucleic Acids Res 40 (Database issue):D1047–D1054.

Lips EH, Gaborieau V, McKay JD, Chabrier A, Hung RJ, Boffetta P, Hashibe M, Zaridze D, Szeszenia-Dabrowska N, Lissowska J, Rudnai P, Fabianova E, Mates D, Bencko V, Foretova L, Janout V, Field JK, Liloglou T, Xinarianos G, McLaughlin J, Liu G, Skorpen F, Elvestad MB, Hveem K, Vatten L, Study E, Benhamou S, Lagiou P, Holcátová I, Merletti F, Kjaerheim K, Agudo A, Castellsagué X, Macfarlane TV, Barzan L, Canova C, Lowry R, Conway DI, Znaor A, Healy C, Curado MP, Koifman S, Eluf-Neto J, Matos E, Menezes A, Fernandez L, Metspalu A, Heath S, Lathrop M, Brennan P (2010) Association between a 15q25 gene variant, smoking quantity and tobacco-related cancers among 17 000 individuals. Int J Epidemiol 39:563–577.

Liu B, Li W, Li Y, Wang Z, Li H, Liu P, Fu J (2009) Protective effects of N-acetylcysteine in isoproterenol-induced myocardium injury in rats. Mol Biol Rep 36:761–765.

López-Moreno JA, Echeverry-Alzate V, Bühler KM (2012) The genetic basis of the endocannabinoid system and drug addiction in humans. J Psychopharmacol 26:133–143.

Loh E-W, Higuchi S, Matsushita S, Murray R, Chen CK, Ball D (2000) Association analysis of the GABA(A) receptor subunit genes cluster on 5q33-34 and alcohol dependence in a Japanese population. Mol Psychiatry 5:301–307.

Loh E-W, Lane HY, Chen CH, Chang PS, Ku LW, Wang KH, Cheng AT (2006) Glutamate decarboxylase genes and alcoholism in Han Taiwanese men. Alcohol Clin Exp Res 30:1817–1823.

Luo X, Kranzler HR, Zhao H, Gelernter J (2003) Haplotypes at the OPRM1 locus are associated with susceptibility to substance dependence in European-Americans. Am J Med Genet B Neuropsychiatr Genet 120B:97–108.

Luo X, Kranzler HR, Zuo L, Lappalainen J, Yang BZ, Gelernter J (2006) ADH4 gene variation is associated with alcohol dependence and drug dependence in European Americans: results from HWD tests and case-control association studies. Neuropsychopharmacology 31:1085–1095.

Luo X, Kranzler HR, Zuo L, Yang BZ, Lappalainen J, Gelernter J (2005) ADH4 gene variation is associated with alcohol and drug dependence: results from family controlled and population-structured association studies. Pharmacogenet Genomics 15:755–768.

Macgregor S, Lind PA, Bucholz KK, Hansell NK, Madden PA, Richter MM, Montgomery GW, Martin NG, Heath AC, Whitfield JB (2009) Associations of ADH and ALDH2 gene variation with self report alcohol reactions, consumption and dependence: an integrated analysis. Hum Mol Genet 18:580–593.

Maldonado R, Valverde O, Berrendero F (2006) Involvement of the endocannabinoid system in drug addiction. Trends Neurosci 29:225–232.

Matsumoto M, Weickert CS, Akil M, Lipska BK, Hyde TM, Herman MM, Kleinman JE, Weinberger DR (2003) Catechol O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. Neuroscience 116:127–137.

McClellan J, King MC (2010) Genetic heterogeneity in human disease. Cell 141:210–217.

Mechoulam R, Parker LA (2013) The endocannabinoid system and the brain. Annu Rev Psychol 64:21–47.

Mereu M, Tronci V, Chun LE, Thomas AM, Green JL, Katz JL, Tanda G (2013) Cocaine-induced endocannabinoid release modulates behavioral and neurochemical sensitization in mice. Addict Biol doi: 10.1111/adb.12080; [Epub ahead of print].

Miguel-Hidalgo JJ (2009) The role of glial cells in drug abuse. Curr Drug Abuse Rev 2:76–82.

Mizoi Y, Yamamoto K, Ueno Y, Fukunaga T, Harada S (1994) Involvement of genetic polymorphism of alcohol and aldehyde dehydrogenases in individual variation of alcohol metabolism. Alcohol Alcohol 29:707–710.

Montano Loza AJ, Ramirez Iglesias MT, Perez Diaz I, Cruz Castellanos S, Garcia Andrade C, Medina Mora ME, Robles Díaz G, Kershenobich D, Gutierrez Reyes G (2006) Association of alcohol-metabolizing genes with alcoholism in a Mexican Indian (Otomi) population. Alcohol 39:73–79.

Morgan CJ, Badawy AA (2001) Alcohol-induced euphoria: exclusion of serotonin. Alcohol Alcohol 36:22–25.

Mottagui-Tabar S, Faghihi MA, Mizuno Y, Engström PG, Lenhard B, Wasserman WW, Wahlestedt C (2005) Identification of functional SNPs in the 5-prime flanking sequences of human genes. BMC Genomics 6:18.

Mulligan MK, Ponomarev I, Hitzemann RJ, Belknap JK, Tabakoff B, Harris RA, Crabbe JC, Blednov YA, Grahame NJ, Phillips TJ, Finn DA, Hoffman PL, Iyer VR, Koob GF, Bergeson SE (2006) Toward understanding the genetics of alcohol drinking through transcriptome meta-analysis. Proc Natl Acad Sci USA 103:6368–6373.

Munafò M, Clark T, Johnstone E, Murphy M, Walton R (2004) The genetic basis for smoking behavior: a systematic review and meta-analysis. Nicotine Tob Res 6:583–597.

Munafò MR, Timpson NJ, David SP, Ebrahim S, Lawlor DA (2009) Association of the DRD2 gene Taq1A polymorphism and smoking behavior: a meta-analysis and new data. Nicotine Tob Res 11:64–76.

Nestler EJ (2002) Common molecular and cellular substrates of addiction and memory. Neurobiol Learn Mem 78:637–647.

Nestler EJ (2005) Is there a common molecular pathway for addiction? Nat Neurosci 8:1445–1449.

Neumark YD, Friedlander Y, Durst R, Leitersdorf E, Jaffe D, Ramchandani VA, O'Connor S, Carr LG, Li TK (2004) Alcohol dehydrogenase polymorphisms influence alcohol-elimination rates in a male Jewish population. Alcohol Clin Exp Res 28:10–14.

Neville MJ, Johnstone EC, Walton RT (2004) Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Hum Mutat 23:540–545.

Noble EP (2003) D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. Am J Med Genet B Neuropsychiatr Genet 116B:103–125.

Noble EP, Blum K, Ritchie T, Montgomery A, Sheridan PJ (1991) Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. Arch Gen Psychiatry 48:648–654.

Noble EP, Gottschalk LA, Fallon JH, Ritchie TL, Wu JC (1997) D2 dopamine receptor polymorphism and brain regional glucose metabolism. Am J Med Genet 74:162–166.

Oertel BG, Kettner M, Scholich K, Renné C, Roskam B, Geisslinger G, Schmidt PH, Lötsch J (2009) A common human micro-opioid receptor genetic variant diminishes the receptor signaling efficacy in brain regions processing the sensory information of pain. J Biol Chem 284:6530–6535.

Oota H, Pakstis AJ, Bonne-Tamir B, Goldman D, Grigorenko E, Kajuna SL, Karoma NJ, Kungulilo S, Lu RB, Odunsi K, Okonofua F, Zhukova OV, Kidd JR, Kidd KK (2004) The evolution and population genetics of the ALDH2 locus: random genetic drift, selection, and low levels of recombination. Ann Hum Genet 68 (Pt2):93–109.

Orio L, Edwards S, George O, Parsons LH, Koob GF (2009) A role for the endocannabinoid system in the increased motivation for cocaine in extended-access conditions. J Neurosci 29:4846–4857.

Osier M, Pakstis AJ, Kidd JR, Lee JF, Yin SJ, Ko HC, Edenberg HJ, Lu RB, Kidd KK (1999) Linkage disequilibrium at the ADH2 and ADH3 loci and risk of alcoholism. Am J Hum Genet 64:1147–1157.

Osier MV, Pakstis AJ, Goldman D, Edenberg HJ, Kidd JR, Kidd KK (2002) A proline-threonine substitution in codon 351 of ADH1C is common in Native Americans. Alcohol Clin Exp Res 26:1759–1763.

Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, Volpicelli JR, O'Brien CP (2003) A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. Neuropsychopharmacology 28:1546–1552.

Palmer RH, McGeary JE, Francazio S, Raphael BJ, Lander AD, Heath AC, Knopik VS (2012) The genetics of alcohol dependence: advancing towards systems-based approaches. Drug Alcohol Depend 125:179–191.

Park BL, Kim JW, Cheong HS, Kim LH, Lee BC, Seo CH, Kang TC, Nam YW, Kim GB, Shin HD, Choi IG (2013) Extended genetic effects of ADH cluster genes on the risk of alcohol dependence: from GWAS to replication. Hum Genet 132:657–668.

Park CS, Park SY, Lee CS, Sohn JW, Hahn GH, Kim BJ (2006) Association between alcoholism and the genetic polymorphisms of the GABAA receptor genes on chromosome 5q33-34 in Korean population. J Korean Med Sci 21:533–538.

Patnala R, Clements J, Batra J (2013) Candidate gene association studies: a comprehensive guide to useful in silico tools. BMC Genet 14:39.

Pava MJ, Woodward JJ (2012) A review of the interactions between alcohol and the endocannabinoid system: implications for alcohol dependence and future directions for research. Alcohol 46:185–204.

Peng GS, Yin SJ (2009) Effect of the allelic variants of aldehyde dehydrogenase ALDH2*2 and alcohol dehydrogenase ADH1B*2 on blood acetaldehyde concentrations. Hum Genomics 3:121–127.

Pertwee RG (2006) The pharmacology of cannabinoid receptors and their ligands: an overview. Int J Obes (Lond) 30 (Suppl. 1):S13–S18.

Piomelli D (2003) The molecular logic of endocannabinoid signaling. Nat Rev Neurosci 4:873–884.

Piomelli D (2004) The endogenous cannabinoid system and the treatment of marijuana dependence. Neuropharmacology 47 (Suppl. 1):359–367.

Pohjalainen T, Rinne JO, Någren K, Lehikoinen P, Anttila K, Syvälahti EK, Hietala J (1998) The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. Mol Psychiatry 3:256–260.

Ponce G, Hoenicka J, Jiménez-Arriero MA, Rodríguez-Jiménez R, Aragüés M, Martín-Suñé N, Huertas E, Palomo T (2008) DRD2 and ANKK1 genotype in alcohol-dependent patients with psychopathic traits: association and interaction study. Br J Psychiatry 193:121–125.

Ponce G, Pérez-González R, Aragüés M, Palomo T, Rodríguez-Jiménez R, Jiménez-Arriero MA, Hoenicka J (2009) The ANKK1 kinase gene and psychiatric disorders. Neurotox Res 16:50–59.

Preuss UW, Ridinger M, Rujescu D, Samochowiec J, Fehr C, Wurst FM, Koller G, Bondy B, Wodarz N, Debniak T, Grzywacz A, Soyka M, Zill P (2011) Association of ADH4 genetic variants with alcohol dependence risk and related phenotypes: results from a larger multicenter association study. Addict Biol 16:323–333.

Quertemont E (2004) Genetic polymorphism in ethanol metabolism: acetaldehyde contribution to alcohol abuse and alcoholism. Mol Psychiatry 9:570–581.

Ramensky V, Bork P, Sunyaev S (2002) Human non-synonymous SNPs: server and survey. Nucleic Acids Res 30:3894–3900.

Ratsma JE, van der Stelt O, Schoffelmeer AN, Westerveld And A, Boudewijn Gunning W (2001) P3 event-related potential, dopamine D2 receptor A1 allele, and sensation-seeking in adult children of alcoholics. Alcohol Clin Exp Res 25:960–967.

Ray LA, Hutchison KE (2004) A polymorphism of the mu-opioid receptor gene (OPRM1) and sensitivity to the effects of alcohol in humans. Alcohol Clin Exp Res 28:1789–1795.

Ray LA, Hutchison KE (2007) Effects of naltrexone on alcohol sensitivity and genetic moderators of medication response: a double-blind placebo-controlled study. Arch Gen Psychiatry 64:1069–1077.

Reimers MA, Riley BP, Kalsi G, Kertes DA, Kendler KS (2012) Pathway based analysis of genotypes in relation to alcohol dependence. Pharmacogenomics J 12:342–348.

Ritchie T, Noble EP (1996) [3H]naloxone binding in the human brain: alcoholism and the TaqI A D2 dopamine receptor polymorphism. Brain Res 718:193–197.

Rivera-Meza M, Quintanilla ME, Tampier L, Mura CV, Sapag A, Israel Y (2010) Mechanism of protection against alcoholism by an alcohol dehydrogenase polymorphism: development of an animal model. FASEB J 24:266–274.

Rommelspacher H, Smolka M, Schmidt LG, Samochowiec J, Hoehe MR (2001) Genetic analysis of the mu-opioid receptor in alcohol-dependent individuals. Alcohol 24:129–135.

Saccone NL, Culverhouse RC, Schwantes-An TH, Cannon DS, Chen X, Cichon S, Giegling I, Han S, Han Y, Keskitalo-Vuokko K, Kong X, Landi MT, Ma JZ, Short SE, Stephens SH, Stevens VL, Sun L, Wang Y, Wenzlaff AS, Aggen SH, Breslau N, Broderick P, Chatterjee N, Chen J, Heath AC, Heliövaara M, Hoft NR, Hunter DJ, Jensen MK, Martin NG, Montgomery GW, Niu T, Payne TJ, Peltonen L, Pergadia ML, Rice JP, Sherva R, Spitz MR, Sun J, Wang JC, Weiss RB, Wheeler W, Witt SH, Yang BZ, Caporaso NE, Ehringer MA, Eisen T, Gapstur SM, Gelernter J, Houlston R, Kaprio J, Kendler KS, Kraft P, Leppert MF, Li MD, Madden PA, Nöthen MM, Pillai S, Rietschel M, Rujescu D, Schwartz A, Amos CI, Bierut LJ (2010) Multiple independent loci at chromosome 15q25.1 affect smoking quantity: a meta-analysis and comparison with lung cancer and COPD. PLoS Genet 6: doi: 10.1371/journal.pgen.1001053.

Saccone NL, Saccone SF, Goate AM, Grucza RA, Hinrichs AL, Rice JP, Bierut LJ (2008) In search of causal variants: refining disease association signals using cross-population contrasts. BMC Genet 9:58.

Saccone NL, Saccone SF, Hinrichs AL, Stitzel JA, Duan W, Pergadia ML, Agrawal A, Breslau N, Grucza RA, Hatsukami D, Johnson EO, Madden PA, Swan GE, Wang JC, Goate AM, Rice JP, Bierut LJ (2009a) Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. Am J Med Genet B Neuropsychiatr Genet 150B:453–466.

Saccone NL, Wang JC, Breslau N, Johnson EO, Hatsukami D, Saccone SF, Grucza RA, Sun L, Duan W, Budde J, Culverhouse RC, Fox L, Hinrichs AL, Steinbach JH, Wu M, Rice JP, Goate AM, Bierut LJ (2009b) The CHRNA5-CHRNA3-CHRNB4 nicotinic receptor subunit gene cluster affects risk for nicotine dependence in African-Americans and in European-Americans. Cancer Res 69:6848–6856.

Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PA, Breslau N, Johnson EO, Hatsukami D, Pomerleau O, Swan GE, Goate AM, Rutter J, Bertelsen S, Fox L, Fugman D, Martin NG, Montgomery GW, Wang JC, Ballinger DG, Rice JP, Bierut LJ (2007) Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. Hum Mol Genet 16:36–49.

Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L, Buhot MC, Hen R (1994) Enhanced aggressive behavior in mice lacking 5-HT1B receptor. Science 265:1875–1878.

Schacht JP, Selling RE, Hutchison KE (2009) Intermediate cannabis dependence phenotypes and the FAAH C385A variant: an exploratory analysis. Psychopharmacology (Berl) 203:511–517.

Serý O, Didden W, Mikes V, Pitelová R, Znojil V, Zvolský P (2006) The association between high-activity COMT allele and alcoholism. Neuro Endocrinol Lett 27:231–235.

Shabalina SA, Zaykin DV, Gris P, Ogurtsov AY, Gauthier J, Shibata K, Tchivileva IE, Belfer I, Mishra B, Kiselycznyk C, Wallace MR, Staud R, Spiridonov NA, Max MB, Goldman D, Fillingim RB, Maixner W, Diatchenko L (2009) Expansion of the human mu-opioid receptor gene architecture: novel functional variants. Hum Mol Genet 18:1037–1051.

Sham PC, Purcell SM (2014) Statistical power and significance testing in large-scale genetic studies. Nat Rev Genet 15:335–346.

Sherva R, Kranzler HR, Yu Y, Logue MW, Poling J, Arias AJ, Anton RF, Oslin D, Farrer LA, Gelernter J (2010) Variation in nicotinic acetylcholine receptor genes is associated with multiple substance dependence phenotypes. Neuropsychopharmacology 35:1921–1931.

Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF (2002) A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci USA 99:8394–8399.

Sun HF, Chang YT, Fann CS, Chang CJ, Chen YH, Hsu YP, Yu WY, Cheng AT (2002) Association study of novel human serotonin 5-HT(1B) polymorphisms with alcohol dependence in Taiwanese Han. Biol Psychiatry 51:896–901.

Sunyaev S, Ramensky V, Koch I, Lathe W 3rd, Kondrashov AS, Bork P (2001) Prediction of deleterious human alleles. Hum Mol Genet 10:591–597.

Syvänen AC (2001) Accessing genetic variation: genotyping single nucleotide polymorphisms. Nat Rev Genet 2:930–942.

Tabakoff B, Saba L, Printz M, Flodman P, Hodgkinson C, Goldman D, Koob G, Richardson HN, Kechris K, Bell RL, Hübner N, Heinig M, Pravenec M, Mangion J, Legault L, Dongier M, Conigrave KM, Whitfield JB, Saunders J, Grant B, Hoffman PL; WHO/ISBRA Study on State and Trait Markers of Alcoholism (2009) Genetical genomic determinants of alcohol consumption in rats and humans. BMC Biol 7:70.

Tenhunen J, Salminen M, Jalanko A, Ukkonen S, Ulmanen I (1993) Structure of the rat catechol-O-methyltransferase gene: separate promoters are used to produce mRNAs for soluble and membrane-bound forms of the enzyme. DNA Cell Biol 12:253–263.

Thomasson HR, Beard JD, Li TK (1995) ADH2 gene polymorphisms are determinants of alcohol pharmacokinetics. Alcohol Clin Exp Res 19:1494–1499.

Thompson J, Thomas N, Singleton A, Piggott M, Lloyd S, Perry EK, Morris CM, Perry RH, Ferrier IN, Court JA (1997) D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. Pharmacogenetics 7:479–484.

Tiihonen J, Hallikainen T, Lachman H, Saito T, Volavka J, Kauhanen J, Salonen JT, Ryynänen OP, Koulu M, Karvonen MK, Pohjalainen T, Syvälahti E, Hietala J (1999) Association between the functional variant of the catechol-O-methyltransferase (COMT) gene and type 1 alcoholism. Mol Psychiatry 4:286–289.

Treutlein J, Rietschel M (2011) Genome-wide association studies of alcohol dependence and substance use disorders. Curr Psychiatry Rep 13:147–155.

Trigo JM, Martin-García E, Berrendero F, Robledo P, Maldonado R (2010) The endogenous opioid system: a common substrate in drug addiction. Drug Alcohol Depend 108:183–194.

Turchi C, Piva F, Solito G, Principato G, Buscemi L, Tagliabracci A (2012) ADH4 intronic variations are associated with alcohol dependence: results from an Italian case-control association study. Pharmacogenet Genomics 22:79–94.

Twyman RM, Primrose SB (2003) Techniques patents for SNP genotyping. Pharmacogenomics 4:67–79.

Tyndale RF, Payne JI, Gerber AL, Sipe JC (2007) The fatty acid amide hydrolase C385A (P129T) missense variant in cannabis users: studies of drug use and dependence in Caucasians. Am J Med Genet B Neuropsychiatr Genet 144B:660–666.

Vallés SL, Blanco AM, Pascual M, Guerri C (2004) Chronic ethanol treatment enhances inflammatory mediators and cell death in the brain and in astrocytes. Brain Pathol 14:365–371.

Voisey J, Swagell CD, Hughes IP, Lawford BR, Young RM, Morris CP (2011) A novel SNP in COMT is associated with alcohol dependence but not opiate or nicotine dependence: a case control study. Behav Brain Funct 7:51.

Vrieze SI, Feng S, Miller MB, Hicks BM, Pankratz N, Abecasis GR, Iacono WG, McGue M (2014) Rare nonsynonymous exonic variants in addiction and behavioral disinhibition. Biol Psychiatry 75:783–789.

Vrieze SI, Iacono WG, McGue M (2012) Confluence of genes, environment, development, and behavior in a post genome-wide association study world. Dev Psychopathol 24:1195–1214.

Wang JC, Cruchaga C, Saccone NL, Bertelsen S, Liu P, Budde JP, Duan W, Fox L, Grucza RA, Kern J, Mayo K, Reyes O, Rice J, Saccone SF, Spiegel N, Steinbach JH, Stitzel JA, Anderson MW, You M, Stevens VL, Bierut LJ, Goate AM, COGEND Collaborators, GELCC Collaborators (2009) Risk for nicotine dependence and lung cancer is conferred by mRNA expression levels and amino acid change in CHRNA5. Hum Mol Genet 18:3125–3135.

Wang KS, Liu X, Zhang Q, Pan Y, Aragam N, Zeng M (2011) A meta-analysis of two genome-wide association studies identifies 3 new loci for alcohol dependence. J Psychiatr Res 45:1419–1425.

Wang S, Yang Z, Ma JZ, Payne TJ, Li MD (2014) Introduction to deep sequencing and its application to drug addiction research with a focus on rare variants. Mol Neurobiol 49:601–614.

Wang T, Franke P, Neidt H, Cichon S, Knapp M, Lichtermann D, Maier W, Propping P, Nöthen MM (2001) Association study of the low-activity allele of catechol-O-methyltransferase and alcoholism using a family-based approach. Mol Psychiatry 6:109–111.

Wassenaar CA, Dong Q, Wei Q, Amos CI, Spitz MR, Tyndale RF (2011) Relationship between CYP2A6 and CHRNA5-CHRNA3-CHRNB4 variation and smoking behaviors and lung cancer risk. J Natl Cancer Inst 103:1342–1346.

Wei RR, Zhang MY, Rao HL, Pu HY, Zhang HZ, Wang HY (2012) Identification of ADH4 as a novel and potential prognostic marker in hepatocellular carcinoma. Med Oncol 29:2737–2743.

Wessel J, McDonald SM, Hinds DA, Stokowski RP, Javitz HS, Kennemer M, Krasnow R, Dirks W, Hardin J, Pitts SJ, Michel M, Jack L, Ballinger DG, McClure JB, Swan GE, Bergen AW (2010) Resequencing of nicotinic acetylcholine receptor genes and association of common and rare variants with the Fagerström test for nicotine dependence. Neuropsychopharmacology 35:2392–2402.

Williams MJ, Adinoff B (2008) The role of acetylcholine in cocaine addiction. Neuropsychopharmacology 33:1779–1797.

Xie P, Kranzler HR, Krauthammer M, Cosgrove KP, Oslin D, Anton RF, Farrer LA, Picciotto MR, Krystal JH, Zhao H, Gelernter J (2011) Rare nonsynonymous variants in alpha-4 nicotinic acetylcholine receptor gene protect against nicotine dependence. Biol Psychiatry 70:528–536.

Xie P, Kranzler HR, Krystal JH, Farrer LA, Zhao H, Gelernter J (2014) Deep resequencing of 17 glutamate system genes identifies rare variants in DISC1 and GRIN2B affecting risk of opioid dependence. Addict Biol 19:955–964.

Yin SJ, Chou CF, Lai CL, Lee SL, Han CL (2003) Human class IV alcohol dehydrogenase: kinetic mechanism, functional roles and medical relevance. Chem Biol Interact 143–144:219–227.

Yoshida A, Rzhetsky A, Hsu LC, Chang C (1998) Human aldehyde dehydrogenase gene family. Eur J Biochem 251:549–557.

Zayats T, Yang BZ, Xie P, Poling J, Farrer LA, Gelernter J (2013) A complex interplay between personality domains, marital status and a variant in CHRNA5 on the risks of cocaine, nicotine dependences and cocaine-induced paranoia. PLoS ONE 8:e49368.

Zhang Y, Bertolino A, Fazio L, Blasi G, Rampino A, Romano R, Lee ML, Xiao T, Papp A, Wang D, Sadée W (2007) Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. Proc Natl Acad Sci USA 104:20552–20557.

Zhang Y, Wang D, Johnson AD, Papp AC, Sadée W (2005) Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. J Biol Chem 280:32618–32624. Erratum in: J Biol Chem 280:38888.

Zuo L, Kranzler HR, Luo X, Yang BZ, Weiss R, Brady K, Poling J, Farrer L, Gelernter J (2009) Interaction between two independent CNR1 variants increases risk for cocaine dependence in European Americans: a replication study in family-based sample and population-based sample. Neuropsychopharmacology 34:1504–1513.

Zuo L, Zhang H, Malison RT, Li CS, Zhang XY, Wang F, Lu L, Lu L, Wang X, Krystal JH, Zhang F, Deng HW, Luo X (2013) Rare ADH variant constellations are specific for alcohol dependence. Alcohol Alcohol 48:9–14.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 References of excluded studies

Table S2 Single nucleotide polymorphisms significantly associated with ALCOHOL-related phenotypes

Table S3 Single nucleotide polymorphisms significantly associated with NICOTINE-related phenotypes

Table S4 Single nucleotide polymorphisms significantly associated with CANNABIS-related phenotypes

Table S5 Single nucleotide polymorphisms significantly associated with COCAINE-related phenotypes